R.J. Klijn Bone augmentation for oral and maxillofacial applications 2012

# BONE AUGMENTATION

## for oral and maxillofacial applications

R.J. Klijn

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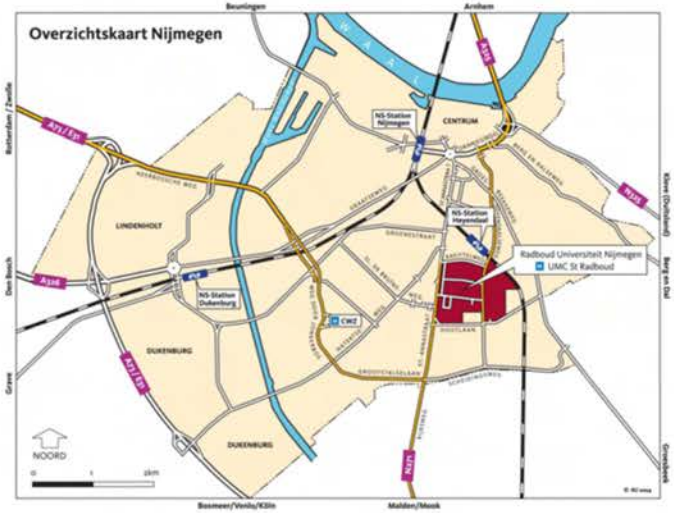


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Jonne Doorduin
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# STELLINGEN

behorende bij het proefschrift

# BONE AUGMENTATION

**for oral and maxillofacial applications**

**R.J. Klijn**

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# STELLINGEN

1. De biologische prestatie van botvervangende materialen in botaugmentatie procedures is gerelateerd aan materiaaleigenschappen, de omgeving waarin het materiaal wordt aangebracht en de individuele condities van de patiënt. *(dit proefschrift)*

2. Op basis van meta-analyse van histologisch onderzoek na sinusbodem-elevatie procedures, moet het autoloog bottransplantaat worden beschouwd als gouden standaard. *(dit proefschrift)*

3. Het creëren van porositeit in calcium fosfaat cement draagt bij aan een verbeterde degradatie met als gevolg een verhoogde mate van botingroei in het materiaal. *(dit proefschrift)*

4. Osteoinductief tricalciumfosfaat en poreus calcium fosfaat cement, zoals beschreven in dit proefschrift, zijn beide geschikte botvervangers voor sinusbodemelevatie procedures in de kliniek. *(dit proefschrift)*

5. He who studies medicine without books sails an uncharted sea, but he who studies medicine without patients does not go to sea at all.
*(William Osler, 1849-1919)*

6. Veel mensen missen ten gevolge van een verkeerd gekozen opleiding veel levensgeluk.

7. Spelfouten vallen vaak meer op dan denkfouten.

8. Door de opkomst van facebook gaat het tegenwoordig bij vriendschappen meer over aantallen dan over inhoud.

9. Tijd wordt vaak verward met prioriteit.

10. De stelligheid waarmee een mening verkondigd wordt is omgekeerd evenredig aan de kans op repliek.

11. Kritiek is altijd welkom, maar komt zelden op het juiste moment.

# BONE AUGMENTATION

for oral and maxillofacial applications

# COLOFON

Thesis Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, with summary in Dutch

**Bone augmentation for oral and maxillofacial applications**

The work described in this thesis was performed at the Departments of Implantology & Periodontology (Head: prof. dr. G.J. Meijer) and Biomaterials (Head: prof. dr. J.A. Jansen) Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

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# BONE AUGMENTATION

## for oral and maxillofacial applications

**PROEFSCHRIFT**

ter verkrijging van de graad van doctor

aan de Radboud Universiteit Nijmegen

op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,

volgens besluit van het college van decanen

in het openbaar te verdedigen

op vrijdag 4 mei 2012

om 13.00 uur precies

door

**Reinder Jan Klijn**

Geboren op 6 april 1984
te Callantsoog

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# BONE AUGMENTATION

## for oral and maxillofacial applications

**DOCTORAL THESIS**

to obtain the degree of doctor

from Radboud University Nijmegen

on the authority of the Rector Magnificus prof. mr. S.C.J.J. Kortmann,

according to the decision of the Council of Deans

to be defended in public

on Friday May 4, 2012

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by

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Born April 6, 1984
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*Voor mijn ouders*

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# CHAPTER 01

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Introduction and objective of the study

# INTRODUCTION

Bone tissue is the most frequently used tissue for transplantation in orthopedic and oral and maxillofacial surgical procedures. Bony defects in the oral and maxillofacial area can be the result of trauma, cancer, infection and congenital disease, but also induced by the loss of teeth. Despite increased oral healthcare and prevention over the last decades, full edentulism is still observed for around 4% of people in the age of 20 to 64 in the United States of America.[1] Moreover, partial edentulism percentages, especially loss of (pre)molars, are even much higher.[1] Resorption of original alveolar bone will occur directly after tooth loss because of the loss of mechanical loading.[2;3] Additionally, this process may as well continue for years eventually resulting in severe atrophic jaw bones.[1-4]

Dentists and oral and maxillofacial surgeons have thought of ways to replace missing teeth and thereby maintain the original alveolar ridge dimensions over time. Treatment options for tooth loss have evolved from traditional fixed or removable (partial) dentures to implant supported constructions in the last few decades. Dental implants are titanium screws that can be placed into the lower or upper jaw (mandible and maxilla, respectively) at the side of lost teeth. Treatment with dental implants has several benefits over traditional treatment options, including a positive effect on the decline in original alveolar bone mass.[5;6] To allow implant placement, sufficient quantity and quality of the existing jawbone are a prerequisite. In most cases, substantial resorption of the original alveolar ridge has already taken place in the time period between tooth loss and dental implant placement. In these complex cases with a decreased alveolar bone volume, the insertion of dental implants is compromised because the atrophic bone tissue does not allow adequate primary fixation.[7-9] In view of this, maxillary bone resorption rates are even increased compared to those of the mandible.[10] Furthermore, maxillary bone tissue is also often of inferior quality.[11] Additionally, in the posterior maxillary (pre)molar region, the presence of the maxillary sinus may even further compromise dental implant placement. The maxillary sinus is located superior to the roots of the (pre)molars and after tooth removal continuously expands because of pneumatization, which further contributes to the reduction of alveolar bone dimensions.[12] Consequently, because of tooth loss and consequent loss of mechanical loading, both alveolar ridge resorption and maxillary sinus pneumatization are accelerated, often resulting in inadequate bone volumes for dental implant placement.

In order to overcome the problem of reduced alveolar bone quantity and quality several bone augmentation techniques have been described.[13] These reconstructive procedures can be performed prior to, or simultaneously with implant placement to ensure implant supported constructions with a long-term

prognosis.[13;14] In view of this, socket preservation, horizontal and vertical ridge augmentation and maxillary sinus floor augmentation procedures, all result in adequate reconstruction of the alveolar ridge.[13]

**Oral and maxillofacial anatomy**

The soft tissues that give structure to the human face have a foundation on the cranium and facial bones. The most important facial bones include the nasal bone, zygoma, mandible and maxilla as shown in Figure 1a. The mandible is the largest and strongest bone of the facial bones and consists of the corpus mandible and a left and right mandibular ramus. Teeth are situated in the mandibular alveolar ridge. The maxilla consists of the corpus maxillae, zygomatic, palatine and frontal processes and the maxillary alveolar ridge, the latter in which the upper jaws teeth are located. Beside bony structures also teeth are part of to the foundation and by supporting the lips and cheeks, providing an aesthetically acceptable appearance. Both teeth in the lower and upper jaw contribute to the preservation of bony oral and maxillofacial structures.

The maxillary sinus is the largest of the paranasal sinuses and is located in the corpus maxillae (Figure 1b). It is surrounded by bony structures, such as the sinus floor which consists of the alveolar process of the maxilla and the sinus roof which consists of the orbit floor. At the (possible) edentulous stage in life, sinus volume will increase, thereby reducing a large part of the alveolar ridge. Moreover, this volume increase may even result in only a very thin alveolar bone wall on both the lateral and occlusal sides. The maxillary sinuses are part of the respiratory system and the lining consists of a layer of loose connective tissue, a surface layer of ciliated columnar cells and sub-epithelial mucous secreting serous cells. Underneath the connective tissue and immediately adjacent to the bone is the periosteum, all together named the Schneiderian membrane. This membrane might have a transport function for fluids like pus and mucus towards the ostium. The ostium is positioned in the superior medial wall and opens into the semilunar hiatus. This location provides the possibility to perform a sinus augmentation procedure without interfering with normal sinus function.

**Bone augmentation procedures**

Alveolar ridge deformities are classified according to their morphology and severity (Figure 2).[11;15] The predictability of a reconstructive augmentation procedure is influenced by the horizontal and vertical extent of the edentulous alveolar ridge. Defects that exhibit both a horizontal and vertical component are less favorable for pre-implant surgical procedures because of insufficient initial bone volume.[13] Several augmentation techniques have been described in literature, including distraction osteogenesis, guided bone regeneration and onlay and inlay bone augmentation.[13] Moreover, all of these procedures have

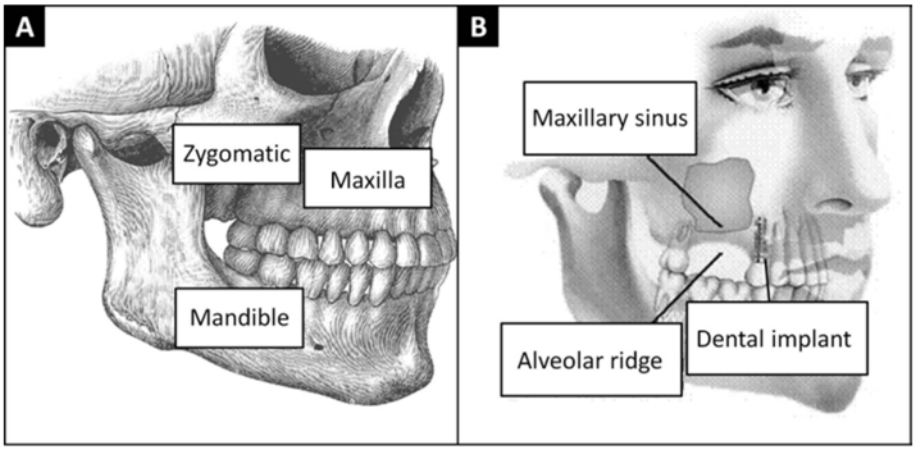


Figure 1: Oral and maxillofacial anatomy

demonstrated high and comparable levels of long-term dental implant survival.[16] On the other hand, a relatively high complication rate has been reported for distraction osteogenesis and guided bone regeneration.[16-18] Taking this into consideration, autologous bone onlay or inlay augmentation must still be considered the favorite bone augmentation technique.

Bone reconstructive procedures have extensively been described for augmentation and filling up of extraction sockets in order to preserve alveolar ridge dimensions after tooth removal.[13] Moreover, if peri-apical bone or socket walls are damaged, bone grafts are used to preserve the original shape of the alveolar ridge.[13] Although preservation of alveolar bone has been described, critical-sized alveolar ridge defects in the horizontal and vertical dimensions are still a very common cause of compromised implant placement.[7-9] Ridge augmentation is a procedure that can recapture the natural contour of the jawbone and gums. Applied augmentation materials must be rigidly fixed to the bony surface underneath the gums.[13] In addition, the augmented area may be protected with (non)-resorbable membranes to seclude this area, prevent soft tissue ingrowth and hence ensure a successful outcome.[13] Furthermore, another appealing option for vertical dimension reconstruction and dental implant placement in the posterior maxilla was introduced in the 1980s by Boyne and James and Tantum and was called maxillary sinus floor augmentation (Figure 3).[19;20]

Maxillary sinus floor augmentation represents a technique based on the elevation of the Schneiderian membrane from the sinus floor, after which this created area is filled with an augmentation material. In order to lift the Schneiderian membrane, the maxillary sinus must be exposed by a modified posterior Caldwell-Luc operation. Caldwell-Luc procedures were described as a surgical method with specific application for clearing an infected or blocked

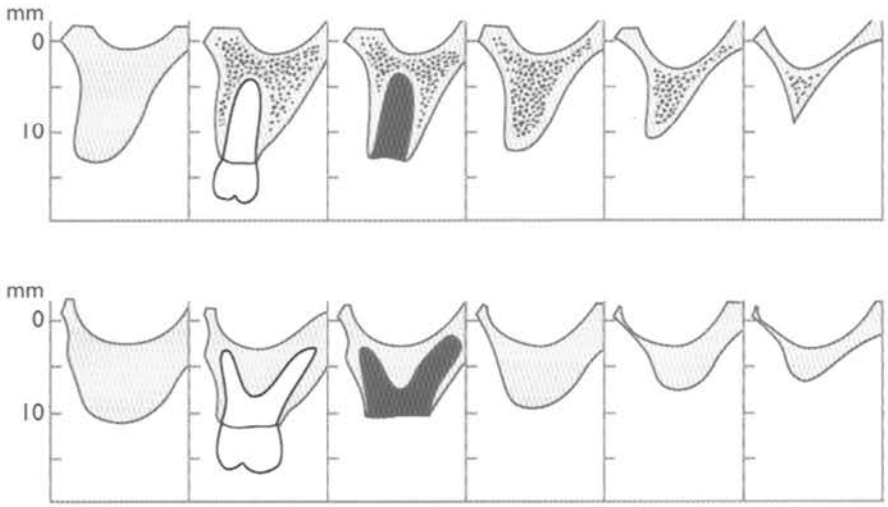


Figure 2: Alveolar ridge resorption

airway caused by sinusitis or in case of malignancies present in the sinus cavity.[21] The modified surgical procedure consists of the creation of a bony window in the lateral maxillary wall after which the Schneiderian membrane can carefully be elevated.[22] In view of sinus floor augmentation, this newly created space can subsequently be filled with an augmentation material. If sufficient bone quantity and quality is obtained, dental implants can directly or after a certain healing time be installed in the posterior maxilla.[23]

Since these early reports in the 1980s, several modifications have been made regarding the surgical technique.[13] In addition to the modified posterior Caldwell-Luc procedure, various other versions of maxillary sinus floor augmentation surgery have evolved over time, each successful in different cases. As such, Summers introduced the crestal approach in 1994, in which osteotomes are used to fracture the sinus floor after which bone grafts can be introduced through the osteotome holes.[24;25] However, the absence of direct visual inspection of the Schneiderian membrane and the grafted area is a major disadvantage of this technique. Although this technique, as well as other inventive approaches including the balloon or hydropneumatic approach, have been described, the original augmentation procedure has been refined and is still the most frequently used method.[13] In addition, the efficacy and predictability of the original maxillary sinus floor augmentation procedure have been determined in numerous studies.[14] Successful outcome can be defined as the elevation of the maxillary sinus floor in order to provide an adequate space and mass of the jaw bone for safe and successful dental implant placement. At this moment, it can be concluded that maxillary sinus floor augmentation surgery is one of the most reliable procedures in pre-implant surgery.

**Bone and bone substitute materials**

Bone tissue and bone substitute materials have been extensively used for augmentation procedures in oral and maxillofacial applications. For such augmentation materials, three different biological processes are associated with successful bone regeneration and augmentation: osteogenesis, osteoinduction and osteoconduction (see Table 1 for definitions). Osteogenesis is the formation and development of bone, an osteogenic graft is derived or composed of tissue involved in the natural growth or repair of bone. Osteoinduction is the stimulation of osteogenesis, osteoinductive grafts can be used to enhance bone regeneration and may cause bone grow into an area where it is normally not found. Osteoconduction provides a physical matrix or scaffold suitable for the deposition of new bone. Osteoconductive grafts guide and allow bone growth and apposition from existing bony surfaces but do not induce bone formation itself. Ideally for clinical applications, degradation of these augmentation materials is in optimal balance with bone formation, which means that the space that becomes available during material degradation is directly filled up by newly-formed bone tissue.

Augmentation materials can be categorized into four main groups:

- autologous bone grafts;
- allografts (harvested from human cadavers);
- xenografts (harvested from nonhuman species); and
- alloplasts (synthetic materials).

Autologous bone is still considered as the gold standard for augmentation procedures owing to its excellent clinical results in the past decades.[14;26-30] Moreover, autologous bone provides a satisfactory source of osteogenic cells without the risk of transmission of diseases or reduced biocompatibility.[30] Furthermore, it is preferred because of its osteoinductive and osteoconductive nature. Relative large volumes of autologous bone can be harvested from the iliac crest, smaller amounts can be obtained from the oral cavity.[30] Unfortunately, harvesting of such an autologous bone graft is associated with serious drawbacks. The major disadvantage is that donor site surgery requires a prolonged operating time and may cause severe donor site morbidity.[14;29] Furthermore, there is only a limited amount of autologous bone available that can be used for oral and maxillofacial reconstructive procedures.

Allografts and xenografts have been used as alternatives to autologous bone.[14;27-30] A number of review articles concluded that with these alternatives to autologous bone, excellent bone augmentation results and a high long-term implant survival are feasible.[14;28-31] However, these alternatives have the potential for severe disease transmission.[14] Because an allograft is obtained from living donors having bone removed during surgery or from cadaveric donors, viral

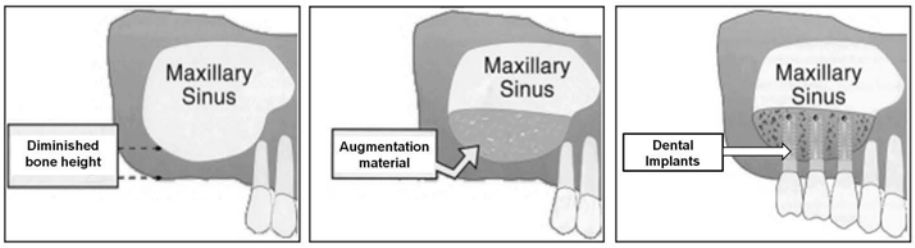

Figure 3: Maxillary sinus floor augmentation surgery and dental implant placement

infections (e.g. hepatitis and HIV) are causes for concern.[14] Moreover, transmission of infectious particles (prions), which may possibly results in Creuzfeldt-Jacob disease, have been reported.[32] However, it must be noted that the risk of disease transmission is almost negligible if these biological materials are adequately processed, including demineralization and lyophilization.[33] Remarkable is the fact that the most common type of graft infections are of bacterial origin, which is typically caused from contamination of the graft or the wound during the surgical procedure.

During the last three decades, a large number of synthetic alloplasts have been developed as alternatives for biologically-derived bone grafts.[14;27-31;34] The major advantages of these alloplastic materials compared to other augmentation materials are their safety, costs and unlimited off the shelf availability. However, in order to allow application in a clinical situation, these synthetic materials need to fulfill a number of criteria. The most important are biocompatibility, biodegradability and easy handling.

The majority of alloplasts consist of the same components as normal bone tissue, evidenced by the large proportion of synthetic bone substitutes that are based on ceramics (e.g. calcium phosphate, CaP). As such, ceramic materials have a long history as bone substitute materials as they show an excellent biocompatible and osteoconductive behavior. However, current research efforts focus further on changing biomaterials from a biologically passive osteoconductive role to one in which the properties of the material will induce the process of tissue regeneration.[35] Moreover, osteoinduction of a bone substitute material has been described to be useful for speeding up the process of appositional bone growth and may offer a solution for patients with a compromised bone forming capacity.[35] Early attempts to enhance bone growth by using biologically active factors, such as bone morphogenetic proteins (BMPs), showed promising results.[36] Moreover, an increasing amount of studies demonstrated product safety with BMP-2 in bone augmentation procedures.[37-40] However, such growth factors are a very expensive treatment option and yet clear dosages are unknown. Moreover, the in vivo delivery of soluble molecules such as BMPs is often inefficient. An increasing number of reports demonstrated that bone

| Requirement | Definition |
|---|---|
| Bioactivity | The ability to form a direct chemical bond with bone and thus a uniquely strong biomaterial interface |
| Biocompatibility | The ability to perform with an appropriate host response in a specific situation |
| Biodegradability | The ability to gradually vanish and leave space for new tissue growth without hindering the regeneration process |
| Osteoconduction | The capacity to guide bone-forming tissue on a surface or down into pores |
| Osteogenesis | The formation and development of bone tissue |
| Osteogenic | Graft that is derived or composed of tissue involved in the natural growth or repair of bone |
| Osteoinduction | The ability to induce bone formation by influencing the differentiation or maturation of stem cells into bone-forming cells |

Table 1: Definitions

formation can also be induced by porous calcium phosphate materials.[41] The underlying mechanism leading to bone induction by alloplasts remains largely unknown. However, the osteoinductive potential of biomaterials can be controlled by tailoring material characteristics such as chemical composition, surface topography, and geometry, which in turn affect resorption rate and cell-material interactions.[35] In addition, osteoinductive materials have been described to be a safe and cheap alternative to the use of autologous bone grafts or more expensive growth factors in reconstructive procedures.[35]

In order to improve the clinical handling properties of alloplastic bone substitutes, Brown and Chow were the first to describe a successful attempt in making self-hardening calcium phosphate cement (CPC).[42;43] Such injectable bone substitute materials allow optimal defect filling and easy shaping. CPCs consist of a mixture of calcium phosphates which can be applied as a paste that usually consolidates at the application site due to precipitation or exothermic reactions.[42] Although numerous reports on *in vitro* and *in vivo* investigations dealing with these CaP cements have been published, there are still some major problems to overcome. CPCs, unlike blocks or granules, have a solid structure characterized by a limited porosity resulting in decreased degradation rates.[42;43] As a solution for this problem, macroporosity can be induced by a large number of strategies. Promising methods are foaming agents which are released during setting or the incorporation of degradable microspheres to induce a controlled and delayed CPC porosity for appositional bone growth.[44-46]

# OBJECTIVE OF THE STUDY

To date, no clear evidence or guideline exists for the use of autologous bone or bone substitute materials for reconstructive augmentation procedures in oral and maxillofacial applications. Moreover, no clear evidence is yet identified that should prompt a clinician to prefer the one or the other in an individual patient. Furthermore, the influence of other clinical factors such as patient specific bone conditions, healing time or the use of a (non)-resorbable membrane on the biological result is also mainly unknown. In order to enhance clinical and biological properties of bone substitute materials, innovative research attempts focus on osteoinductivity, biodegradability and handling properties. The general hypothesis on the use of bone substitutes for oral and maxillofacial applications is that their performance is predominantly related to bone substitute material properties, recipient site characteristics, and patient conditions. In view of this, the overall aim of the research described in this thesis was to assess the value of various types of augmentation materials for oral and maxillofacial bone procedures. Moreover, the secondary aim was to evaluate the possible future clinical use of innovative synthetic augmentation materials in preclinical animal models. In more detail, the research described in this thesis is associated to the following scientific questions:

- Which approach is the best when using autologous bone grafts for human maxillary sinus floor augmentation surgery?

- Which augmentation material is best for application in human maxillary sinus floor augmentation procedures and at what graft healing time is required?

- Are there any patient specific parameters that predict the outcome of maxillary sinus floor augmentation surgery using autologous bone grafts?

- How is the *in vitro* and *in vivo* degradation and biological performance of instantaneous and delayed porous CaP cement?

- How do CaP-PLGA composites and osteoinductive microstructured CaP granules perform in a sheep maxillary sinus floor augmentation model and can they be suggested for further clinical bone augmentation procedures?

# REFERENCES


1. Dye B.A., Tan S., Smith V., Lewis B.G., Barker L.K., Thornton-Evans G., Eke P.I., Beltran-Aguilar E.D., Horowitz A.M., and Li C.H. Trends in oral health status: United States, 1988-1994 and 1999-2004. Vital Health Statistics 11, 1, 2007.
2. Pietrokovski J. and Massler M. Alveolar ridge resorption following tooth extraction. Journal of Prosthetic Dentistry 17, 21, 1967.
3. Farina R., Pramstraller M., Franceschetti G., Pramstraller C., and Trombelli L. Alveolar ridge dimensions in maxillary posterior sextants: a retrospective comparative study of dentate and edentulous sites using computerized tomography data. Clinical Oral Implants Research 22, 1138, 2011.
4. Pramstraller M., Farina R., Franceschetti G., Pramstraller C., and Trombelli L. Ridge dimensions of the edentulous posterior maxilla: a retrospective analysis of a cohort of 127 patients using computerized tomography data. Clinical Oral Implants Research 22, 54, 2011.
5. Lindquist L.W., Carlsson G.E., and Jemt T. A prospective 15-year follow-up study of mandibular fixed prostheses supported by osseointegrated implants. Clinical results and marginal bone loss. Clinical Oral Implants Research 7, 329, 1996.
6. Schropp L., Wenzel A., Kostopoulos L., and Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. International Journal of Periodontics & Restorative Dentistry 23, 313, 2003.
7. Eufinger H., Konig S., Eufinger A., and Machtens E. Significance of the height and width of the alveolar ridge in implantology in the edentulous maxilla. Analysis of 95 cadaver jaws and 24 consecutive patients. Mund Kiefer Gesichtschirurgie 3 Suppl 1, S14, 1999.
8. Eufinger H., Konig S., and Eufinger A. The role of alveolar ridge width in dental implantology. Clinical Oral Investigations 1, 169, 1997.
9. Tepper G., Haas R., Zechner W., Krach W., and Watzek G. Three-dimensional finite element analysis of implant stability in the atrophic posterior maxilla: a mathematical study of the sinus floor augmentation. Clinical Oral Implants Research 13, 657, 2002.
10. Klemetti E. A review of residual ridge resorption and bone density. Journal of Prosthetic Dentistry 75, 512, 1996.
11. Norton M.R. and Gamble C. Bone classification: an objective scale of bone density using the computerized tomography scan. Clinical Oral Implants Research 12, 79, 2001.
12. Sharan A. and Madjar D. Maxillary sinus pneumatization following extractions: a radiographic study. International Journal of Oral & Maxillofacial Implants 23, 48, 2008.
13. McAllister B.S. and Haghighat K. Bone augmentation techniques. Journal Periodontology 78, 377, 2007.
14. Nkenke E. and Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clinical Oral Implants Research 20 Suppl 4, 124, 2009.
15. Cawood J.I. and Howell R.A. A classification of the edentulous jaws. International Journal of Oral & Maxillofacial Surgery 17, 232, 1988.
16. Nevins M., Mellonig J.T., Clem D.S., III, Reiser G.M., and Buser D.A. Implants in regenerated bone: long-term survival. International Journal of Periodontics & Restorative Dentistry 18, 34, 1998.
17. McAllister B.S. and Gaffaney T.E. Distraction osteogenesis for vertical bone augmentation prior to oral implant reconstruction. Periodontology 2000 33, 54, 2003.
18. Tamimi F., Torres J., Gbureck U., Lopez-Cabarcos E., Bassett D.C., Alkhraisat M.H., and Barralet J.E. Craniofacial vertical bone augmentation: a comparison between 3D printed monolithic monetite blocks and autologous onlay grafts in the rabbit. Biomaterials 30, 6318, 2009.
19. Boyne P.J. and James R.A. Grafting of the maxillary sinus floor with autogenous marrow and bone. Journal of Oral Surgery 38, 613, 1980.
20. Tatum H., Jr. Maxillary and sinus implant reconstructions. Dental Clinics of North America 30, 207, 1986.

21. Macbeth R. Caldwell, Luc, and their operation. Laryngoscope 81, 1652, 1971.
22. Boyne P.J. and James R.A. Grafting of the maxillary sinus floor with autogenous marrow and bone. Journal of Oral Surgery 38, 613, 1980.
23. Tatum H., Jr. Maxillary and sinus implant reconstructions. Dental Clinics of North America 30, 207, 1986.
24. Summers R.B. A new concept in maxillary implant surgery: the osteotome technique. Compendium 15, 152, 1994.
25. Pjetursson B.E., Rast C., Bragger U., Schmidlin K., Zwahlen M., and Lang N.P. Maxillary sinus floor elevation using the (transalveolar) osteotome technique with or without grafting material. Part I: Implant survival and patients' perception. Clinical Oral Implants Research 20, 667, 2009.
26. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. Sinus floor augmentation surgery using autologous bone grafts from various donor sites: a meta-analysis of the total bone volume. Tissue Engineering Part B Reviews 16, 295, 2010.
27. Wallace S.S. and Froum S.J. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Annual Periodontology 8, 328, 2003.
28. Tong D.C., Rioux K., Drangsholt M., and Beirne O.R. A review of survival rates for implants placed in grafted maxillary sinuses using meta-analysis. International Journal of Oral & Maxillofacial Implants 13, 175, 1998.
29. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. A Meta-Analysis of Histomorphometric Results and Graft Healing Time of Various Biomaterials Compared to Autologous Bone Used as Sinus Floor Augmentation Material in Humans. Tissue Engineering Part B Reviews 16, 493, 2010.
30. Kaufman E. Maxillary sinus elevation surgery: an overview. Journal of Esthetic and Restorative Dentistry 15, 272, 2003.
31. Merkx M.A., Maltha J.C., and Stoelinga P.J. Assessment of the value of anorganic bone additives in sinus floor augmentation: a review of clinical reports. International Journal of Oral & Maxillofacial Surgery 32, 1, 2003.
32. Brown P., Preece M.A., and Will R.G. "Friendly fire" in medicine: hormones, homografts, and Creutzfeldt-Jakob disease. Lancet 340, 24, 1992.
33. Gomes K.U., Carlini J.L., Biron C., Rapoport A., and Dedivitis R.A. Use of allogeneic bone graft in maxillary reconstruction for installation of dental implants. Journal of Oral and Maxillofacial Surgery 66, 2335, 2008.
34. Wheeler S.L. Sinus augmentation for dental implants: the use of alloplastic materials. Journal of Oral and Maxillofacial Surgery 55, 1287, 1997.
35. Yuan H., Fernandes H., Habibovic P., de B.J., Barradas A.M., de R.A., Walsh W.R., van Blitterswijk C.A., and de Bruijn J.D. Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. Proceedings of the National Academy of Sciences USA 107, 13614, 2010.
36. Urist M.R. Bone: formation by autoinduction. Science 150, 893, 1965.
37. Wang J.C., Alanay A., Mark D., Kanim L.E., Campbell P.A., Dawson E.G., and Lieberman J.R. A comparison of commercially available demineralized bone matrix for spinal fusion. European Spine Journal 16, 1233, 2007.
38. Drosos G.I., Kazakos K.I., Kouzoumpasis P., and Verettas D.A. Safety and efficacy of commercially available demineralised bone matrix preparations: a critical review of clinical studies. Injury 38 Suppl 4, S13, 2007.
39. Bormann N., Pruss A., Schmidmaier G., and Wildemann B. In vitro testing of the osteoinductive potential of different bony allograft preparations. Archives of Orthopaedic and Trauma Surgery 130, 143, 2010.
40. Garrison K.R., Donell S., Ryder J., Shemilt I., Mugford M., Harvey I., and Song F. Clinical effectiveness and cost-effectiveness of bone morphogenetic proteins in the non-healing of fractures and spinal fusion: a systematic review. Health Technology Assessment 11, 1, 2007.
41. LeGeros R.Z. Calcium phosphate-based osteoinductive materials. Chemical Reviews 108, 4742, 2008.
42. Bohner M. Design of ceramic-based cements and putties for bone graft substitution. European

Cells and Materials 20, 1, 2010.

43. Wagoner Johnson A.J. and Herschler B.A. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. Acta Biomaterialia 7, 16, 2011.
44. del Real R.P., Wolke J.G., Vallet-Regi M., and Jansen J.A. A new method to produce macropores in calcium phosphate cements. Biomaterials 23, 3673, 2002.
45. del Real R.P., Ooms E., Wolke J.G., Vallet-Regi M., and Jansen J.A. In vivo bone response to porous calcium phosphate cement. Journal of Biomedical Materials Research part A 65, 30, 2003.
46. Liao H., Walboomers X.F., Habraken W.J., Zhang Z., Li Y., Grijpma D.W., Mikos A.G., Wolke J.G., and Jansen J.A. Injectable calcium phosphate cement with PLGA, gelatin and PTMC microspheres in a rabbit femoral defect. Acta Biomaterialia 7, 1752, 2011.

# CHAPTER 02

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# CHAPTER 02

## Sinus floor augmentation surgery using autologous bone grafts from various donor sites: A meta-analysis of the total bone volume

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# INTRODUCTION

Placement of dental implants requires the presence of adequate bone height en width. In case of lack of bone volume, additional surgical techniques are needed to generate primary implant stability.[1] If extensive alveolar defects are present, onlay or inlay grafting procedures have been advised.[1-7] To allow implant placement in the lateral part of the maxilla, sinus floor augmentation has become a routine procedure,[8-11] resulting in an implant survival rate of 90 percent for 3 to 5 years.[7,12] In this procedure, first a small window is created in the lateral wall of the maxilla. Subsequently the sinus epithelium is elevated and the created space filled with a grafting material. For sinus floor augmentations autologous bone is the most common used material and as such still considered to be the gold standard,[7,13] although numerous alternative materials have been used with variable results.

Both intra oral and extra oral sites can be considered as donor sites,[14] As such, the chin and retromolar region, as also the iliac crest, calvarium, tibia and rib have been described,[15,16] An advantage of intraoral donor sites is that the graft can be harvested under local anesthesia. However, the amount of bone that can be gained is limited. If larger bone volumes are needed, the iliac crest is the most common used donor site. Alternatives, such as bone substitutes do not provide the cellular elements necessary for osteogenesis, as they are only osteoconductive.[17,18] For bone transplants, in addition to donor site, other variables may influence the final outcome.[9] For example, some studies advise to apply a resorbable or non-resorbable barrier membrane over the sinus graft osteotomy site.[19-22]

Till now, no studies have been published that evaluated histological and histomorphometric data from a large amount of patients inventorying these variables. To answer the question which approach is the best in using autologous bone grafts after sinus floor augmentation surgery, a meta-analysis was conducted.

| | |
|---|---|
| "Sinus Augmentation" OR "Sinus Lift" | 428 hits |
| AND (Human OR Patient OR Clinical) | 401 hits |
| AND (Histology OR Histomorphometric OR Histomorphometry | 147 hits |

Table 1: Literature search

# MATERIALS AND METHODS

### Search protocol and selection of articles

An online and manual search was conducted of the Medline database from January 1995 till April 2009 using the PubMed search machine entering the following search terms: "(Maxillary) Sinus augmentation or (Maxillary) Sinus lift" and "human or clinical or patient" and "histology or histomorphometry or histomorphometric". A hand search was performed in the following journals: Clinical Oral Implant Research, International Journal of Oral and Maxillofacial Implants, International Journal of Periodontics and Restorative Dentistry, and the Journal of Periodontology. Additionally, the references of the retrieved articles were searched. The results were limited to humans as well as articles published in the English literature. Articles were regarded eligible if they met the following inclusion criteria: the target population comprised adult patients suffering from maxillary atrophy; the intervention was maxillary sinus floor augmentation using an autologous bone graft; histomorphometric data about bone formation were present. Each potentially appropriate study included at least two patients per specific treatment in whom an autologous bone graft was used as the only augmentation material after conducting the maxillary sinus floor elevation procedure.

Articles were excluded if they reported only one patient or if histomorphometric data were absent. Each retrieved citation was reviewed by two independently working reviewers. Most articles were excluded on basis of information provided by the title or abstract. If the citation could not be excluded unequivocally, the complete report was obtained by the two reviewers. Any disagreement between them was resolved by consensus. The two reviewers extracted from each eligible article all pertinent information regarding patients' demographics, maxillary sinus floor augmentation surgery, and outcome data. Demographic data included age, gender, follow-up rate and duration. Also, the number of patients, the number of treated maxillary sinuses, possible usage of collagen or non-resorbable membranes, further operative data and all results from histomorphometry were noted. To ensure consistency of the results for the included studies clear definitions of outcome were defined. For example, "Total Bone Volume" (TBV) was based on histomorphometric data as a percentage of the whole field of view.

Subsequently, the included studies were carefully analyzed concerning data on age, gender, immediate or delayed implant placement, membrane usage, biopsy time, histological section thickness, graft site, particulated versus block grafting technique and TBV. Where adequate data were available, subgroups of

similar interventions with respect to, for example, surgical techniques, donor site, membrane and biopsy time, were isolated and subjected to backward linear regression to identify them as possible sources of covariance.

**Meta-analysis**

Linear regression, a form of meta analysis, was performed to determine the effect of the independent variables: 'age', 'donor site location', 'block graft', 'particulated graft', presence of a 'resorbable barrier membrane' over the lateral window, 'simultaneous and delayed' implant placement, 'biopsy time' and 'histological section thickness' on the histomorphometric outcome after autologous bone grafting in maxillary sinus floor augmentation. The amount of TBV was used as the dependent variable. To evaluate the influence of 'biopsy time' on the histomorphometric data outcome, the subgroups were divided into 3 different groups: 0-4 months, 4-6.5 months and longer than 6.5 months. To reduce the initial model, stepwise backward linear regression was applied with a threshold for the P value of above 0.1 for removing a variable from the model. The overall averages were controlled for study characteristics and can therefore be considered meta-effect sizes. Thus, more powerful estimates of the true effect sizes than those derived in a single study under a given single set of assumptions and conditions can be given.

**Included studies and donor sites**

The basis search provided 147 titles for consideration (Table 1). 25 articles met our inclusion criteria. Frequently (17), autologous bone volume percentages were extracted from comparative experiments in which they were used as a control group. The majority of these 25 articles were prospective controlled studies (21), two randomized clinical trials, one pilot study followed by one case series. Only two prospective randomized clinical trials met the inclusion criteria (Table 2). Of the 25 articles derived from the main search, 15 discussed the use of autogenous bone from the iliac crest for augmentation of the maxillary sinus floor. The use of chin bone as grafting material was found in six of the 25 articles. The use of intra oral bone, the chin excluded, was investigated in seven out of 25 articles.

| Author | Reference | Type | Graft and Donor site | Total number of treated patients |
|---|---|---|---|---|
| Barone et al. (2005) | 22 | RCT | Iliac | 18 |
| Consolo et al. (2007) | 23 | CT | Iliac | 16 |
| Crespi et al. (2007) | 21 | CT | Iliac or Miscellaneous | 16 |
| Crespi et al. (2009) | 36 | CT | Miscellaneous | 15 |
| Gerressen et al. (2009) | 43 | CT | Iliac | 15 |
| Groeneveld et al. (1999) | 46 | CS | Iliac | 12 |
| Hallman et al. (2002) | 37 | CT | Miscellaneous | 21 |
| John and Wenz (2004) | 47 | CT | Chin | 38 |
| Le Lorc'h-Bukiet et al. (2005) | 35 | CT | Chin or Miscellaneous | 24 |
| Lorenzetti et al. (1998) | 27 | CT | Iliac or Chin | 13 |
| Lundgren et al. (1996) | 14 | CT | Chin or Miscellaneous | 10 |
| Pejrone et al. (2002) | 26 | CT | Iliac | 13 |
| Peleg et al. (2004) | 20 | CT | Miscellaneous | 156 |
| Raghoebar et al. (2005) | 24 | CT | Iliac | 5 |
| Scarano et al. (2006) | 34 | CT | Miscellaneous | 94 |
| Suba et al. (2006) | 48 | CT | Iliac | 17 |
| Szabo et al. (2001) | 44 | CT | Iliac | 4 |
| Szabo et al. (2005) | 49 | RCT | Iliac | 20 |
| Tadjoedin et al. (2000) | 28 | CT | Iliac | 10 |
| Thor et al. (2007) | 25 | CT | Iliac | 11 |
| Turunen et al. (2004) | 50 | CT | Iliac | 17 |
| van den Bergh et al. (2000) | 42 | CPS | Iliac | 6 |
| Zerbo et al. (2003) | 19 | CT | Chin | 19 |
| Zerbo et al. (2004) | 29 | CT | Chin | 9 |
| Zijderveld et al. (2005) | 33 | CT | Chin | 10 |

Table 2: Overview of the analyzed articles (RCT: Randomized Clinical Trail; CT: Clinical Trial; CPS: Clinical pilot study; CS: Case Series)

| Author | Ref. | Number of Sinuses | Mean Age | Particulated or Block | Simultaneous (1) or delayed (2) implant placement | Mean biopsy time (Months) | Slice thickness | Mean Total Bone Volume (%) |
|---|---|---|---|---|---|---|---|---|
| Pejrone et al. (2002) | 26 | 26 | 57 | Block | 1 | 0 | 7 | 59.3±6.3 |
| Lorenzetti et al. (1998) | 27 | 8 | 51.75 | Both | 1 | 0 | 8 | 60 |
| Thor et al. (2007) | 25 | 11 | 55 | Particulated | 2 | 3 | 12.5 | 11±3 |
| Raghoebar et al. (2005) | 24 | 5 | 58.4 | Both | 2 | 3 | 2 | 41.1±8.3 |
| Consolo et al. (2007) | 23 | 2 | 47 | Particulated | 2 | 4 | 5 | 26±5.2 |
| Tadjoedin et al. (2000) | 28 | 3 | 54 | Particulated | 2 | 4 | 5 | 40.94±3.32 |
| Consolo et al. (2007) | 23 | 2 | 47 | Particulated | 2 | 5 | 5 | 29.2±4 |
| Crespi et al. (2007) | 21 | 6 | 51.4 | Particulated | 2 | 5 | 30 | 34±21 |
| Tadjoedin et al. (2000) | 28 | 3 | 54 | Particulated | 2 | 5 | 5 | 42.24±4.48 |
| Barone et al. (2005) | 22 | 18 | 46.7 | Particulated | 2 | 5 | 50 | 70±19.9 |
| Gerressen et al. (2009) | 43 | 15 | 54.9 | Particulated | 2 | 5.2 | ? | 29.35±4.04 |
| Gerressen et al. (2009) | 43 | 15 | 54.9 | Particulated | 2 | 5.2 | ? | 37.87±12.18 |
| Thor et al. (2007) | 25 | 11 | 55 | Particulated | 2 | 6 | 12.5 | 13±6 |
| Groeneveld et al. (1999) | 46 | 3 | 55 | Particulated | 2 | 6 | 5 | 26.2±5.9 |
| van den Bergh et al. (2000) | 42 | 3 | 50 | Particulated | 2 | 6 | 3 | 26.6±5.9 |
| Consolo et al. (2007) | 23 | 2 | 47 | Particulated | 2 | 6 | 5 | 29 |
| Szabo et al. (2001) | 44 | 4 | 52 | Particulated | 2 | 6 | ? | 37.05±8.66 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Szabo et al. (2005) | 49 | 20 | 52 | Particulated | 2 | 6 | 5 | 38.34±7.4 |
| Tadjoedin et al. (2000) | 28 | 3 | 54 | Particulated | 2 | 6 | 5 | 43.65±2.38 |
| Lorenzetti et al. (1998) | 27 | 8 | 51.75 | Both | 2 | 6 | 8 | 53 |
| Pejrone et al. (2002) | 26 | 26 | 57 | Block | 2 | 6 | 7 | 54.1±6.8 |
| Suba et al. (2006) | 48 | 17 | 52 | Particulated | 2 | 6.5 | 5 | 34.7±11.86 |
| Consolo et al. (2007) | 23 | 2 | 47 | Particulated | 2 | 7 | 5 | 20 |
| Turunen et al. (2004) | 21 | 17 | 50 | Both | 2 | 7 | 20 | 25.1±7.2 |
| Turunen et al. (2004) | 50 | 17 | 50 | Both | 2 | 12 | 20 | 25.1±6.3 |
| Pejrone et al. (2002) | 26 | 26 | 57 | Block | 2 | 12 | 7 | 63.9±8.7 |

Table 3: Iliac bone grafting

| Author | Ref. | Number of Sinuses | Mean Age | Particulated or Block | Simultaneous (1) or delayed (2) implant placement | Mean biopsy time (Months) | Slice thickness | Mean Total Bone Volume (%) |
|---|---|---|---|---|---|---|---|---|
| Lundgren et al. (1996) | 14 | 10 | 53 | Block | 1 | 0 | 10 | 58±19 |
| Lorenzetti et al. (1998) | 27 | 3 | 52.33 | Particulated | 1 | 0 | 8 | 65.6 |
| Lorenzetti et al. (1998) | 27 | 3 | 51.33 | Block | 1 | 0 | ? | 65.6 |
| Zerbo et al. (2003) | 19 | 5 | 39.2 | Block | 2 | 3 | 5 | 39.38 |
| Zerbo et al. (2003) | 19 | 6 | 32.33 | Block | 2 | 4 | 5 | 39.78 |
| Lorenzetti et al. (1998) | 27 | 3 | 51.33 | Block | 2 | 4 | 8 | 62.9 |
| John and Wenz (2004) | 47 | 4 | 52 | Particulated | 1/2 | 5 | 50 | 53.5±2.52 |
| Lundgren et al. (1996) | 14 | 10 | 53 | Particulated | 2 | 6 | 10 | 40±12 |
| Zerbo et al. (2003) | 19 | 3 | 33.67 | Block | 2 | 6 | 5 | 40.9 |
| Zerbo et al. (2004) | 29 | 2 | 53.8 | Particulated | 2 | 6 | 5 | 41±10 |
| Zijderveld et al. (2005) | 33 | 3 | 53.83 | Particulated | 2 | 6 | 5 | 41±10 |
| Lorenzetti et al. (1998) | 27 | 3 | 52.33 | Particulated | 2 | 10.6 | 8 | 69.3 |
| Lundgren et al. (1996) | 14 | 10 | 53 | Particulated | 2 | 12 | 10 | 48±10 |

Table 4: Intra oral chin bone grafting

| Author | Ref. | Number of Sinuses | Mean Age | Particulated or Block | Simultaneous (1) or delayed (2) implant placement | Mean biopsy time (Months) | Slice thickness | Mean Total Bone Volume (%) |
|---|---|---|---|---|---|---|---|---|
| Lundgren et al. (1996) | 14 | 10 | 53 | Particulated | 1 | 0 | 10 | 45±15 |
| Peleg et al. (2004) | 20 | 97 | | Particulated | ? | 4.5 | ? | 31.5 |
| Le Lorc'h-Bukiet et al. (2005) | 35 | 24 | 59 | Particulated | 2 | 5 | 4 | 49.4±18.4 |
| Crespi et al. (2007) | 21 | 10 | 51.4 | Particulated | 2 | 5 | 30 | 69.7±16.1 |
| Crespi et al. (2009) | 36 | 15 | 54.2 | Particulated | 2 | 5 | 30 | 78.4±16.72 |
| Scarano et al. (2006) | 34 | 16 | 61 | Particulated | 2 | 6 | 30 | 40.1±3.2 |
| Hallman et al. (2002) | 37 | 11 | 54 | Particulated | 2 | 12.5 | 10 | 37.3±31.3 |

Table 5: Intra oral miscellaneous bone grafting

| Author | Ref. | Number of Sinuses | Mean Age | Donor Site | Membrane | Mean biopsy time (Months) | Slice thickness | Mean Total Bone Volume (%) |
|---|---|---|---|---|---|---|---|---|
| Zerbo et al. (2003) | 19 | 5 | 39.2 | Chin | Collagen | 3 | 5 | 39.38 |
| Zerbo et al. (2003) | 19 | 6 | 32.33 | Chin | Collagen | 4 | 5 | 39.78 |
| Peleg et al. (2004) | 20 | 97 | | Intra oral | Collagen | 4.5 | ? | 31.5 |
| Crespi et al. (2007) | 21 | 6 | 51.4 | Iliac crest | Collagen | 5 | 30 | 34±21 |
| Crespi et al. (2007) | 21 | 10 | 51.4 | Intra oral | Collagen | 5 | 30 | 69.7±16.1 |
| Barone et al. (2005) | 22 | 18 | 46.7 | Iliac crest | Collagen | 5 | 50 | 70±19.9 |
| Zerbo et al. (2003) | 19 | 3 | 33.67 | Chin | Collagen | 6 | 5 | 40.9 |

Table 6: Membrane use

# RESULTS

**Donor site**

***Iliac***

Bone grafts were harvested from the anterior as well as the posterior iliac crest. From these articles, adequate data were found to discern 26 individual subgroups describing 273 sinus floor augmentation procedures (Table 3). Histomorphometric results from biopsies were described for sinus floor augmentation procedures up to 12 months after initial surgery. Bone blocks, particulated bone grafts or a combination of both were used. Several authors combined autogenous bone with Platelet Rich Plasma (PRP),[23-25] but those subgroups were left out of consideration. At the moment of sinus floor augmentation, bone grafts showed a TBV of approximately 60%,[26,27] varying from 32.5 to 78.4%. After backward linear regression significant evidence was present, that the use of iliac bone grafts will result in a lower amount of TBV than intra oral bone grafting.

***Intra oral bone: chin***

From the included articles 13 different subgroups could be identified describing the use of chin bone. In seven of them autologous bone was used as particulate and in six as bone blocks, with a total of 65 sinus floor augmentation procedures (Table 4). These studies evaluated biopsies taken both immediately after sinus floor augmentation as after a healing period with a range of 3-12 months. At the moment of initial surgery, a TBV of 58-65% was presented.[14,27] After healing (3-12 months) the TBV varied between 39.38% to 69.3%. After backward linear regression, significant evidence was found, that the use of chin bone grafts will have a positive effect on the amount of TBV compared to iliac bone grafts. Chin bone grafts increase the TBV with 10.7% at a confidence interval of [1.3%,20.1%] and a p-value of 0.026 (Table 8).

***Intra oral bone: miscellaneous***

Seven subgroups could be discerned within the included articles describing the histomorphometric results from 183 augmented sinuses grafted with intra oral bone, excluded the chin. As intra oral donor sites the anterior maxillary wall, the zygomatico-maxillary buttress, the lateral mandibular body and ramus were chosen (Table 5). Although not of intra-oral origin, also parietal bone was included in this group. All studies described results from particulated bone grafts, no bone blocks were used. Biopsies were examined immediately after initial surgery and after a healing period of 4.5-12.5 months. At the moment of initial augmentation surgery, bone grafts presented a TBV of 45%.[14] After healing, TBV differed within a range of 39.38% to 69.3% over time. After statistical analysis there was significant evidence that the use of intra oral bone grafts had

| Model 1 | | Unstandardized | Significancy | 95% Confidence Interval for B | |
|---|---|---|---|---|---|
| | | B | *p* | Lower bound | Upper bound |
| | (Constant) | 35.8 | 0.305 | -34.1 | 105.7 |
| | Age centered | 1.019 | 0.083 | -0.139 | 2.178 |
| | Intra oral chin | 10.6 | 0.049 | 0.05 | 21.1 |
| | Particulate | -24.7 | 0.021 | -45.5 | -3.9 |
| | Block | -8.4 | 0.372 | -27.3 | 10.5 |
| | Implantology | 8.7 | 0.599 | -24.5 | 41.9 |
| | Biopsy 0-4 months | 0.9 | 0.878 | -10.7 | 12.5 |
| | Biopsy >6.5 months | 2.0 | 0.752 | -10.8 | 14.8 |
| | Membrane | 3.8 | 0.689 | -15.6 | 23.4 |
| | Intra oral | 10.9 | 0.111 | -2.7 | 24.34 |
| | Slice thickness | 0.422 | 0.067 | -0.030 | 0.875 |

Table 7: Full linear regression model, $R^2$=0.475; adjusted $R^2$=0.316.
Dependent Variable: Total Bone Volume

a positive effect on the amount of TBV as compared to iliac bone grafts. Usage of intra oral bone results into an increase of 13.5% bone with a confidence interval of [2.1%,24.9%] and a p-value of 0.021. No significant differences between the various intra oral donor sites, including chin, bone could be found.

***Membrane***

The use of a barrier membrane over the lateral wall to protect the autologous bone graft was investigated in 4 out of the 25 articles. Donor sites for the bone grafts were the iliac crest, chin and other intra oral bone sites. From these articles seven subgroups could be determined describing the histomorphometric results from 145 augmented sinuses (Table 6). Patients had a mean age between 32.3 and 51.4 years. In all subgroups a collagen barrier membrane was placed over the graft according to the principal of guided bone regeneration.[19-22] Furthermore, Peleg et al. also described the use of lypophilized dura mater for such purpose. Core biopsies were obtained after a healing period of 3 to 6 months with a mean TBV of 31,5% to 70%. After backward linear regression there was no significant evidence that the use of a resorbable membrane over the lateral window had a positive or negative effect on the amount of TBV.

**Statistical analysis**

The 11 variables entered into the regression model are shown in Table 7 along with their p-values. The R-square for the full model (all 11 variables) was 0.475. After removal of non-significant variables with backward selection, five significant variables remained in the model. All five variables showed a significance of $p<0.1$ with a model R-square of 0.453, thus explaining the variation in TBV as described in the histomorphometric results. In Table 8, the results of the linear regression analysis of the predicting variables on TBV are given. Only the variables 'patients age', 'intra oral chin bone', 'intra oral miscellaneous bone', 'particulated' grafts and 'histological section thickness' remained in the model. All effects have been corrected for different parameters inside the model by backward regression. After linear backward regression a reference value for total bone volume of 46.5 was found.

***Patients age***

For each year the patient is above 50, an increase of 0.84% of TBV is to be expected with a confidence interval of [0.07%,1.61%]

***Donor site***

Usage of a intra oral chin bone graft increases the TBV with 10.7% with a confidence interval of [1.3%,20.1%], while usage of bone from miscellaneous intra oral sites leads to an increase of 13.5% bone with a confidence interval of [2.1%,24.9%]. Therefore, iliac bone grafts result in a significant lower amount of TBV. Taken into account that the confidence intervals of intra oral and chin bone grafts had a significant overlap, the difference between the donor site regarding amount of TBV was not statistically significant. All data were divided into 3 different biopsy time groups, $0\leq t \leq 4$ months, $4<t\leq 6.5$ months and $t> 6.5$ months. There was no significant evidence that the amount of TBV was influenced by either immediate or delayed implant placement or graft healing time, thus the biopsy time of the samples. No evidence could be found that the use of a resorbable membrane over the lateral window had any effect, positive or negative, on the amount of total bone volume.

***Particulated and block grafting***

Particulation of the bone graft showed a negative effect on TBV, as a decrease of -17.8% was seen with a confidence interval of [-28.2%,-7.5%]. Block grafting had no significant positive or negative influence on the histomorphometric outcome.

***Histological section thickness***

Histological section thickness seemed to be a significant variable to total bone volume in histomorphometric coupes. Every micro-meter increase of section thickness leads to an increase of 0.387% of TBV with a confidence interval of [0.066%,0.708%].

# DISCUSSION

Sinus floor augmentation surgery has become a routine procedure that provides adequate bone volume for placement, stabilization and osteointegration of dental implants. Intra-oral defects, which needed to be reconstructed to allow dental implant placement, are ideal test sites to evaluate the grafted material. Before preparation of the implant bed to install dental implants, a biopsy of the reconstructed area can be easily taken, which implicates no extra burden for the patient.[28,29] Moreover, all surgical procedures, except the extra-oral grafting procedure, can be performed under local anesthesia. The selection of a donor site is often made on considerations driven by the quantity needed for the individual case. In the selected papers various bone substitutes with different results were used to increase the grafting volume. However, bone substitutes do not provide the cellular elements necessary for osteogenesis, as they are solely osteoconductive. Autologous bone grafts are considered to be the gold standard. This conclusion, however, is only based on implant survival, while bone quality in the grafted area is often left out of consideration.[8] The aim of this study was to give a powerful estimate of the true effect of different variables on the TBV in sinus floor augmentation surgery using autologous bone graft derived from various donor sites. Predominantly prospective controlled trials were described (21), only 2 randomized controlled trials met the inclusion

| | Final Model | Unstandardized | Significancy | 95% confidence interval for B | |
|---|---|---|---|---|---|
| | | B | *p* | Lower bound | Upper bound |
| | (Constant) | 46.5 | 0.000 | 36.769 | 56.390 |
| | Age centered | 0.838 | 0.034 | 0.066 | 1.610 |
| | Intra oral chin | 10.7 | 0.026 | 1.3 | 20.1 |
| | Particulate | -17.8 | 0.001 | -28.2 | -7.5 |
| | Intra oral | 13.5 | 0.021 | 2.1 | 24.9 |
| | Slice thickness | 0.387 | 0.020 | 0.066 | 0.708 |

Table 8: Linear backward regression final model, $R^2$=0.453; adjusted $R^2$=0.380.
Dependent Variable: Total Bone Volume

criteria, also a pilot study and one case series was included. Backward linear regression was performed. The dependent variable to assess the quality of the bone graft was solely the TBV because of the general absence of other histomorphometric indices in the studies. Only the variables ´patient age', 'intra oral chin bone', 'intra oral miscellaneous bone', 'particulated grafts' and 'slice thickness´ seemed to have a significant effect and remained in the model.

**Patient age**

In a first glance it seems surprising that, for each year the patient gets older, an increase of 0.84 percent of TBV is to be expected. However, one should realize that bone remodeling, e.g. the quantitative and qualitative changes in bone tissue and in bones themselves, do not only occur during growth but also during normal aging. These changes in the elderly result in a loss of bone mass and bone strength,[30] as a sign of decreased ability to regenerate bone. As the biopsies are generally taken within the first year after the sinus augmentation procedure, we hypothesize that, although speculative, the slight increase of TBV for each year the patient gets older, may be explained by the reduced remodeling capacity of the patient.

**Donor site**

Intra oral grafting from miscellaneous sites, including the chin region, offers the advantage of local, instead of general anesthesia, a limited distance between donor site and augmentation site and the avoidance of cutaneous scars. Although the amount of bone to be gained is rather low, chin bone not only can be grafted in particulate, but even in block form. Predominantly, autologous bone grafts obtained from the iliac crest were investigated. Although several drawbacks were reported, including donor site morbidity and prolonged operation time, a relative larger amount of bone is available that can be harvested in multiple forms (particles, strips and blocks). In this meta-analysis, it is shown that bone grafts from the iliac crest lead to significant lower bone volume than chin bone. Some argue that embryology plays a role; ectomesenchymal mandibular bone grafts survive better than mesenchymal iliac bone grafts in an ectomesenchymal environment as the maxillary sinus.[32] Furthermore, the mechanical stress distribution on the bone graft will be different, or be lost after grafting of the maxillary sinus so resorption may occur more rapidly. In addition, Lundgren et al. concluded that the advantage of inserting implants and performing sinus floor augmentation simultaneously (immediate placement) is that loading and subsequent preservation of the chin bone graft can be initiated earlier.[14] However, they also showed an increased bone volume fraction in the graft throughout the healing time, even though the transplanted bone grafts received no stimulatory loading forces.[14]

It has been shown that also bone grafts, harvested from intra oral sites other than chin, lead to a significant higher TBV as compared to iliac crest bone. Complications related to the harvesting of intra oral bone are seldom reported especially using the shaving technique described by Peleg et al..[20] Scarano et al. compared different materials in maxillary sinus floor augmentation including intra oral autologous bone grafts.[34] Almost all autologous bone particles were completely surrounded by newly formed bone.[34] A low resorption process was present and the graft showed a pattern similar to that of host bone.[34] Le Lorc'h-Bukiet et al. concluded that bone remodeling seems to be more active in the cancellous portion than in the cortical portion.[35] After 10 months of grafting the bone chips were incorporated in newly formed bone and almost completely resorbed.[35] Crespi et al. used autologous bone from the ascending ramus of the mandible. After 5 months biopsies were taken and both lamellar and woven bone was observed.[36] Gene expression profiles revealed expression of certain genes, indicative of osteoblast differentiation and bone formation.[36] Hallman et al. allowed autologous bone graft to heal for an average time of 7,5 months and advocated the placement of extra micro implants to evaluate the bone-implant contact interface without interfering with the healing of standard implants.[37] This technique also made it possible to correlate the histological and histomorphometric findings with the clinical outcome of the standard implants placed in the same area.[37] In 2003 Wallace et al. concluded in a meta-analysis on the survival of endosseous dental implants that membrane utilization is a useful adjunctive therapy that results in an increased survival rate of implant in the grafted maxillary sinus.[9] It was also reported that the increase in implant survival could be explained by the higher percentage of bone.[9,45] Surprisingly, this could not be confirmed by meta-analysis of the four selected papers.[19-22]

**Particulated and block grafting**

After statistical analysis, particulation of the bone graft seems to lead to a significant lower amount of TBV, where there was no such evidence for bone blocks. In literature it is reported that up to 33% of the autologous bone graft can resorb during the initial six months after sinus floor augmentation surgery.[23,26,38,39] These processes of bone resorption and remodeling have a major influence on the clinical outcome and are described to be a continuous problem as this effect of significant initial bone resorption may persist for years. [31,40,41] Zerbo et al. performed sinus augmentation surgery using monocortical blocks of autologous chin bone in order to allow dental implant placement.[19] As a result from biopsies taken at 2.5 and 7 months, the amount of non-vital bone decreased significantly with the time of healing, as it was progressively remodelled into new bone.[19] Zijderveld et al. observed predominantly lamellar bone after a healing period of six months.[33] Lorenzetti et al. performed unilateral

augmentation with bone blocks or particulated bone originating from the chin. In the particulated subgroup the healing period had been reduced since the original compact bone structure had been removed by the particulation process.[27] Chin bone blocks prevailed more over fibrous tissue ingrowth and showed to be more compact than particulated grafts. However, vitality within the cortical component varied to a great extent.[27] The chin bone blocks exhibited an increased bone quantity unlike the particulated chin bone and iliac bone blocks.[27] A remarkable higher number of blood vessels was noted within the particulated bone graft than in block bone grafts.[27] In a study of Consolo et al. biopsies were obtained after four, five, six and seven months after iliac bone grafting. The amount of bone decreased over time, thus resorption was observed.[23] Also these authors state that graft volume resorption seemed to occur during the early phase of healing.[27] Pejrone et al. described that the amount of mineralized tissue was increased after 12 months compared to at baseline and measurements after six months, concluding that bone growth and remodeling has taken place.[26] Furthermore, histomorphometric results from Tadjoedin et al. showed that trabecular bone was present after four months in autologous iliac bone grafts. This bone contained viable osteocytes, was of a mature lamellar type and showed a mature histological appearance.[28] In addition, bone volume continued to increase slightly at five and six months.[28] In a study of Van den Bergh et al. autogenous iliac crest grafts were used for sinus floor augmentation, from which after six months of healing core biopsies were obtained. In all five autogenous grafted sinuses bone appearance was observed similar to normal maxillary bone, clinically as well as histologically.[42] Gerressen et al. performed sinus floor augmentation procedures using grafts from the iliac crest consisting of purely cancellous bone or a composite of cancellous and cortical bone. Both resulted in reliably good bone quality suitable to allow osteointegration of dental implants.[43] The purely cancellous bone graft however seemed to be superior to the mixture, although an average healing time of six months until implant insertion seems to be appropriate for both.[43] Surprisingly, after statistical analysis of the literature, there was no significant evidence of resorption or differences in TBV in time. Szabo et al. stressed that, although bone formation in the surgical area can be influenced by several factors, mainly individual patient factors strongly influence the fate of the various graft materials in the organism.[44]

**Histological section thickness**

Histomorphometry is the most frequently used method to evaluate the structural properties and the amount of TBV in biopsy samples, as obtained for example, after maxillary sinus floor augmentations. Assessment is traditionally in two dimensions, which offers high spatial resolution and high image contrast.

Section thickness, however, seemed to be a significant variable to total bone volume in histomorphometric coupes. In most studies 5-10 micron thick histological sections were used, while in others 10-30 micron or even 50 micron thick sections were prepared. Furthermore, no information was described about the quality or the staining of the histological sections which potentially could also influence the histomorphometric outcome.

**Summary**

In literature no proof is available for an improved implant survival for the various donor sites.[51] However, there are indications that bone harvested from an intra oral donor site will lead to a higher mineralization rate and increased incorporation compared to iliac bone grafting.[17,31] The choice for selecting intra oral or iliac crest is made on a pure clinical basis. If smaller amount of bone is requested, the intra oral region will be selected. As an extra advantage such procedure can be performed under local anesthetics. Are larger bone volumes needed, for example in the case of bilateral sinus augmentation procedures, the iliac crest will be selected as donor site. Application of iliac bone grafts, however, will result in a significant lower TBV as compared to bone grafts harvested from intra oral donor sites. No significant evidence was present, that 'biopsy time', 'immediate or delayed implant placement', 'block grafting' or the 'usage of a resorbable barrier membrane' had a positive or negative influence on the amount of TBV. However, it was reported that membrane utilization will increase implant survival due to the higher percentage of bone.[9,45] Surprisingly, this could not be confirmed by meta analysis of the 4 selected papers.[19-22] Further more patient age and histomorphometric slice thickness were significant variables on total bone volume.

# CONCLUSION

'Age',' intra oral chin grafts', 'intra oral miscellaneous grafts', 'particulate' and 'histological section thickness' were determined as significant variables on the histomorphometric outcome of TBV after sinus floor augmentation surgery using autologous bone. Only particulation of the bone graft leads to a significant lower amount of the TBV, as the others will lead to a significant higher amount of total bone volume. No correlation between TBV and time of graft healing could be found. Bone grafting from the iliac crest resulted in a significantly lower TBV compared to intra oral bone grafting. Although of clinical relevance, in case a considerable amount of bone is needed, such as in augmenting the severely atrophic maxilla, the iliac crest, has still to be considered the gold standard.

# REFERENCES


1. Kaufman E. Maxillary sinus elevation surgery: an overview. Journal of Esthetic and Restorative Dentistry 15, 272, 2003.
2. Boyne P.J. and James R.A. Grafting of the maxillary sinus floor with autogenous marrow and bone. Journal of Oral Surgery 38, 613, 1980.
3. Misch C.E. Maxillary sinus augmentation for endosteal implants: organized alternative treatment plans. International Journal of Oral Implantology 4, 49, 1987.
4. Esposito M., Grusovin M.G., Worthington H.V., and Coulthard P. Interventions for replacing missing teeth: bone augmentation techniques for dental implant treatment. Cochrane Database Syst. Reviews CD003607, 2006.
5. Esposito M., Grusovin M.G., Coulthard P., and Worthington H.V. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. International Journal of Oral & Maxillofacial Implants 21, 696, 2006.
6. Tantum H. Maxillary sinus implant reconstructions. Dentistry Clinical North America 30, 207, 1987.
7. Tong D.C., Rioux K., Drangsholt M., and Beirne O.R. A review of survival rates for implants placed in grafted maxillary sinuses using meta-analysis. International Journal of Oral & Maxillofacial Implants 13, 175, 1998.
8. Merkx M.A., Maltha J.C., and Stoelinga P.J. Assessment of the value of anorganic bone additives in sinus floor augmentation: a review of clinical reports. International Journal of Oral & Maxillofacial Surgery 32, 1, 2003.
9. Wallace S.S. and Froum S.J. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Annual Periodontology 8, 328, 2003.
10. Del Fabbro M., Testori T., Francetti L., and Weinstein R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. International Journal of Periodontics & Restorative Dentistry 24, 565, 2004.
11. Emmerich D., Att W., and Stappert C. Sinus floor elevation using osteotomes: a systematic review and meta-analysis. Journal of Periodontology 76, 1237, 2005.
12. Hurzeler M.B., Kirsch A., Ackermann K.L., and Quinones C.R. Reconstruction of the severely resorbed maxilla with dental implants in the augmented maxillary sinus: a 5-year clinical investigation. International Journal of Oral & Maxillofacial Implants 11, 466, 1996.
13. Jensen O.T., Shulman L.B., Block M.S., and Iacono V.J. Report of the Sinus Consensus Conference of 1996. International Journal of Oral & Maxillofacial Implants 13 Suppl, 11, 1998.
14. Lundgren S., Moy P., Johansson C., and Nilsson H. Augmentation of the maxillary sinus floor with particulated mandible: a histologic and histomorphometric study. International Journal of Oral & Maxillofacial Implants 11, 760, 1996.
15. Nkenke E., Schultze-Mosgau S., Radespiel-Troger M., Kloss F., and Neukam F.W. Morbidity of harvesting of chin grafts: a prospective study. Clinical Oral Implants Research 12, 495, 2001.
16. Nkenke E., Radespiel-Troger M., Wiltfang J., Schultze-Mosgau S., Winkler G., and Neukam F.W. Morbidity of harvesting of retromolar bone grafts: a prospective study. Clinical Oral Implants Research 13, 514, 2002.
17. Thorwarth M., Srour S., Felszeghy E., Kessler P., Schultze-Mosgau S., and Schlegel K.A. Stability of autogenous bone grafts after sinus lift procedures: a comparative study between anterior and posterior aspects of the iliac crest and an intraoral donor site. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics 100, 278, 2005.
18. Ichelmann-Reidy M.E. and Yukn, R.A. Bone replacement grafts. The bone substitutes. Dental Clinics of North America 42, 491, 1998.
19. Zerbo I.R., de Lange G.L., Joldersma M., Bronckers A.L., and Burger E.H. Fate of monocortical bone blocks grafted in the human maxilla: a histological and histomorphometric study. Clinical Oral Implants Research 14, 759, 2003.

20. Peleg M., Garg A.K., Misch C.M., and Mazor Z. Maxillary sinus and ridge augmentations using a surface-derived autogenous bone graft. Journal of Oral and Maxillofacial Surgery 62, 1535, 2004.
21. Crespi R., Vinci R., Cappare P., Gherlone E., and Romanos G.E. Calvarial versus iliac crest for autologous bone graft material for a sinus lift procedure: a histomorphometric study. International Journal of Oral & Maxillofacial Implants 22, 527, 2007.
22. Barone A., Crespi R., Aldini N.N., Fini M., Giardino R., and Covani U. Maxillary sinus augmentation: histologic and histomorphometric analysis. International Journal of Oral & Maxillofacial Implants 20, 519, 2005.
23. Consolo U., Zaffe D., Bertoldi C., and Ceccherelli G. Platelet-rich plasma activity on maxillary sinus floor augmentation by autologous bone. Clinical Oral Implants Research 18, 252, 2007.
24. Raghoebar G.M., Schortinghuis J., Liem R.S., Ruben J.L., van der Wal J.E., and Vissink A. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clinical Oral Implants Research 16, 349, 2005.
25. Thor A., Franke-Stenport V., Johansson C.B., and Rasmusson L. Early bone formation in human bone grafts treated with platelet-rich plasma: preliminary histomorphometric results. International Journal of Oral & Maxillofacial Surgery 36, 1164, 2007.
26. Pejrone G., Lorenzetti M., Mozzati M., Valente G., Schierano G.M. Sinus floor augmentation with autogenous iliac bone block grafts: a histological and histomorphometrical report on the two-step surgical technique. International Journal of Oral & Maxillofacial Surgery 31, 383, 2002.
27. Lorenzetti M., Mozzati M., Campanino P.P., and Valente G. Bone augmentation of the inferior floor of the maxillary sinus with autogenous bone or composite bone grafts: a histologic-histomorphometric preliminary report. International Journal of Oral & Maxillofacial Implants 13, 69, 1998.
28. Tadjoedin E.S., de Lange G.L., Holzmann P.J., Kulper L., and Burger E.H. Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. Clinical Oral Implants Research 11, 334, 2000.
29. Zerbo I.R., Zijderveld S.A., de Boer A., Bronckers A.L., de Lange G., ten Bruggenkate C.M., and Burger E.H. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clinical Oral Implants Research 15, 724, 2004.
30. Kiebzak G.M. Age-related bone changes. Experimental Gerontology 26, 171, 1991.
31. Schlegel K.A., Schultze-Mosgau S., Wiltfang J., Neukam F.W., Rupprecht S., and Thorwarth M. Changes of mineralization of free autogenous bone grafts used for sinus floor elevation. Clinical Oral Implants Research 17, 673, 2006.
32. Koole R. Ectomesenchymal mandibular symphysis bone graft: an improvement in alveolar cleft grafting? Cleft Palate Craniofacial Journal 31, 217, 1994.
33. Zijderveld S.A., Zerbo I.R., van den Bergh J.P., Schulten E.A., and ten Bruggenkate C.M. Maxillary sinus floor augmentation using a beta-tricalcium phosphate (Cerasorb) alone compared to autogenous bone grafts. International Journal of Oral & Maxillofacial Implants 20, 432, 2005.
34. Scarano A., Degidi M., Iezzi G., Pecora G., Piattelli M., Orsini G., Caputi S., Perrotti V., Mangano C., and Piattelli A. Maxillary sinus augmentation with different biomaterials: a comparative histologic and histomorphometric study in man. Implant Dentistry 15, 197, 2006.
35. Le Lorc'h-Bukiet I., Tulasne J.F., Llorens A., and Lesclous P. Parietal bone as graft material for maxillary sinus floor elevation: structure and remodeling of the donor and of recipient sites. Clinical Oral Implants Research 16, 244, 2005.
36. Crespi R., Mariani E., Benasciutti E., Cappare P., Cenci S., and Gherlone E. Magnesium-enriched hydroxyapatite versus autologous bone in maxillary sinus grafting: combining histomorphometry with osteoblast gene expression profiles ex vivo. Journal of Periodontology 80, 586, 2009.
37. Hallman M., Sennerby L., and Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. International Journal of Oral & Maxillofacial Implants 17, 635, 2002.

38. Jensen O.T. and Sennerby L. Histologic analysis of clinically retrieved titanium microimplants placed in conjunction with maxillary sinus floor augmentation. International Journal of Oral & Maxillofacial Implants 13, 513, 1998.
39. Raghoebar G.M., Batenburg R.H., Timmenga N.M., Vissink A., and Reintsema H. Morbidity and complications of bone grafting of the floor of the maxillary sinus for the placement of endosseous implants. Mund Kiefer Gesichtschirurgie 3 Suppl 1, S65, 1999.
40. Verhoeven J.W., Cune M.S., Terlou M., Zoon M.A., and de Putter C. The combined use of endosteal implants and iliac crest onlay grafts in the severely atrophic mandible: a longitudinal study. International Journal of Oral & Maxillofacial Surgery 26, 351, 1997.
41. Triplett R.G., Schow S.R., and Laskin D.M. Oral and maxillofacial surgery advances in implant dentistry. International Journal of Oral & Maxillofacial Implants 15, 47, 2000.
42. van den Bergh J.P., ten Bruggenkate C.M., Groeneveld H.H., Burger E.H., and Tuinzing D.B. Recombinant human bone morphogenetic protein-7 in maxillary sinus floor elevation surgery in 3 patients compared to autogenous bone grafts. A clinical pilot study. Journal of Clinical Periodontology 27, 627, 2000.
43. Gerressen M., Hermanns-Sachweh B., Riediger D., Hilgers R.D., Spiekermann H., and Ghassemi A. Purely cancellous vs. corticocancellous bone in sinus floor augmentation with autogenous iliac crest: a prospective clinical trial. Clinical Oral Implants Research 20, 109, 2009.
44. Szabo G., Suba Z., Hrabak K., Barabas J., and Nemeth Z. Autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevations (2- and 3-dimensional computed tomographic, histologic, and histomorphometric evaluations): preliminary results. International Journal of Oral & Maxillofacial Implants 16, 681, 2001.
45. Tarnow D.P., Wallace S.S., Froum S.J., Rohrer M.D., and Cho S.C. Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: Part 3 of an ongoing prospective study. International Journal of Periodontics & Restorative Dentistry 20, 117, 2000.
46. Groeneveld E.H., van den Bergh J.P., Holzman P., ten Bruggenkate C.M., Tuinzing D.B., and Burger E.H. Histomorphometrical analysis of bone formed in human maxillary sinus floor elevations grafted with OP-1 device, demineralized bone matrix or autogenous bone. Comparison with non-grafted sites in a series of case reports. Clinical Oral Implants Research 10, 499, 1999.
47. John H.D. and Wenz B. Histomorphometric analysis of natural bone mineral for maxillary sinus augmentation. International Journal of Oral & Maxillofacial Implants 19, 199,2004
48. Suba Z., Takacs D., Matusovits D., Barabas J., Fazekas A., and Szabo G. Maxillary sinus floor grafting with beta-tricalcium phosphate in humans: density and microarchitecture of the newly formed bone. Clinical Oral Implants Research 17, 102, 2006
49. Szabo G., Huys L., Coulthard P., Maiorana C., Garagiola U., Barabas J., Nemeth Z., Hrabak K., and Suba Z. A prospective multicentre randomized clinical trial of autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevation: histologic and histomorphometric evaluation. International Journal of Oral & Maxillofacial Implants 20, 371, 2005.
50. Turunen T., Peltola J., Yli-Urpo A., and Happonen R.P. Bioactive glass granules as a bone adjuntive material in maxillary sinus floor augmentation. Clinical Oral Implants Research 15, 135, 2004.
51. Nkenke E. and Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clinical Oral Implants Research 20, 124, 2009.

# CHAPTER 03

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# CHAPTER 03

## A meta-analysis of histomorphometric results and graft healing time of various biomaterials compared to autologous bone used as sinus floor augmentation material in humans

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# INTRODUCTION

Sinus floor augmentation surgery has become a routine procedure to generate primary implant placement and stability in the lateral part of the maxilla, resulting in an implant survival rate of 90 percent for 3 to 5 years.[1-7] Autologous bone is the most common used graft material and, as such, still considered to be the gold standard.[8;9] Unfortunately, harvesting an autologous bone graft is associated with several disadvantages. Donor site surgery requires prolonged operating time and may cause morbidity.[10-14] To avoid serious advents of taking iliac crest bone transplants, such as hypersensitivity,[15] pelvic instability, infection,[14;16] and paraesthesia,[17] the mandibular symphysis has been advocated as an alternative donor site,[13;18-20] however, grafting chin bone may induce complications as well, such as paraesthesia[21] and apical root damage.[12,22] In contrast to intra-oral donor sites, a relative larger amount of bone is available in the iliac crest that can be harvested in multiple forms (particles, strips and blocks).

To overcome the disadvantages of autologous bone grafting in sinus augmentation surgery, various allogenic, xenogenic and alloplastic graft materials or combination of these materials have been tested, followed by variable results.[23] Demineralized Freeze Dried Bone Allograft (DFDBA) and also Mineralized Freeze Dried Bone Allograft (MFDBA) are obtained from cadaver bone which is cleaned and chemically treated.[24] Both have been proven to be biocompatible and osteoconductive[25] and are harvested in the same matter, with the only difference that the DFDBA material undergoes the additional step of decalcification. This also accounts for Anorganic Deproteinized Bovine Bone (ADBB),[26] which is a xenogenic bone graft from which all organic components have been removed,[27] although still small amounts of proteins may be present, including growth factors such as TGF-β and BMP-2.[28] Furthermore alloplastic materials have been investigated. As such, promising results for bioactive glass (BG) composites were reported.[29] Their bioactivity stimulates the reparative process,[30] resulting in a relatively fast bone ingrowth compared to for example hydroxyapatite (HA).[31] HA, either hydrothermally converted from coral or synthetically manufactured, shows a crystalline spatial structure close to that of cortical bone matrix[32] and it is considered to be osteoconductive.[27;33] The degradation of HA is relatively slow and is related to the amount of porosity of the material; it may dissolute at the surface or resorb by the activity of macrophages and multinucleated giant cells.[34;35] Pure-phase beta-tricalcium phosphate (β-TCP), as a derivate of HA, has been shown to be completely resorbable and, in addition, is simultaneously replaced by new bone formation.[36;37] For the sake of completeness, also calcium sulfate, calcium carbonate, hydrogels, (biodegradable) polymers and tissue engineered constructs, either combined with growth factors or cultured cells,

are described in the literature as graft material in human sinus floor augmentation. Except for the selected biomaterial, other variables may also influence the final outcome.[38] For example, some authors advise to apply a resorbable or non-resorbable barrier membrane over the sinus graft osteotomy site,[39-42] or propagate immediate or delayed placement of dental implants. Others recommend a prolonged graft healing time.[7]

It is reported that a higher percentage of bone volume results in a higher implant-bone contact, thereby resulting in a higher implant survival.[38] Furthermore, the percentage of total bone volume (TBV) formed is an important parameter of the performance of a bone graft or bone replacement graft in an augmented sinus.[24;43-45] Till now, no studies have been published which evaluated histological and histomorphometric data related to different biomaterials and their variables from a large group of patients. Therefore, to answer which graft material results in the highest TBV in human sinus floor augmentation surgery and which graft healing time is the most optimal, a meta-analysis was conducted.

| | | (n) |
|---|---|---|
| "Sinus Augmentation" OR "Sinus Lift" | | 428 hits |
| AND (Human OR Patient OR Clinical) | | 401 hits |
| AND (Histology OR Histomorphometric OR Histomorphometry | | 147 hits |
| | | |
| Included | | 64 Articles |
| | Randomized Clinical Trails | 8 Articles |
| | Prospective Clinical Trials | 48 Articles |
| | Case Series | 8 Articles |

Table 1: Literature overview

# MATERIALS AND METHODS

### Search Protocol and Selection of Articles

An online and manual search was conducted of the Medline database from January 1993 till April 2009 using the PubMed search machine entering the following search terms: "(maxillary) sinus augmentation or (maxillary) sinus lift" and "human or clinical or patient" and "histology or histomorphometry or histomorphometric". A hand search was performed in the following journals: Clinical Oral Implant Research, International Journal of Oral and Maxillofacial Implants, International Journal of Periodontics and Restorative Dentistry, and the Journal of Periodontology. As well, the references of the retrieved articles were searched. The results were limited to humans and to articles published in the English literature. Articles were only regarded eligible if they included lateral sinus augmentation surgery in which an autogenic, allogenic, xenogenic or alloplastic graft material, solely or in combination, was placed. Furthermore, histomorphometric data about TBV needed to be present. Effects elucidated by a graft mixture with more than 90% volume of one biomaterial was fully accounted to that specific biomaterial, except if Platelet Rich Plasma (PRP) was added. Each retrieved citation was reviewed by two independently working reviewers. Most articles were excluded on the basis of information provided by the title or abstract. If the citation could not be excluded unequivocally, any disagreement was resolved by consensus. To ensure consistency of the results for the included studies clear definitions of outcome were defined. For example, TBV was based on histomorphometric data as a percentage of the whole field of view. Subsequently, the included studies were carefully analyzed concerning data on 'graft material', 'biopsy time', 'block grafting technique', 'particulated grafting technique', the usage of a '(non)-resorbable membrane' over the lateral window, 'immediate or delayed' implant placement and TBV. Where adequate data were available, subgroups of similar interventions were identified. At least five subgroups, including at least two sinus floor augmentations per group, describing a graft material or combination of graft materials, had to be reported in the literature to include their data in this analysis.

### Meta-Analysis

Linear regression, a form of meta analysis, was performed to determine the effect of the independent variables: 'graft material', 'biopsy time', 'block grafting technique', 'particulated grafting technique', the usage of a '(non)-resorbable membrane' over the lateral window, and 'immediate or delayed' implant placement on the histomorphometric outcome after maxillary sinus floor augmentation. The amount of TBV was used as the dependent variable. To evaluate the general influence of 'biopsy time' on the histomorphometric data outcome,

all data were equally divided into three different groups of time: 0-4.5 months, 4.5-9 months and longer than 9 months. The reference group comprised the use of an autologous bone graft with a biopsy time between 4.5 and 9 months, immediate implant placement and no membrane use. The overall averages were controlled for study characteristics and weighted by study size.

A second linear regression was performed to correct the found TBV for each graft material or combination of graft materials, for 'biopsy time'. All subgroups were divided into three subgroups based on biopsy time: 0-4.5 months, 4.5-9 months and longer than 9 months. The amount of TBV was used as the dependent variable. Independent variables were 'block grafting technique', 'particulated grafting technique', the usage of a '(non)-resorbable membrane' over the lateral window, and 'immediate or delayed implant placement'. The reference group was identical. All effects have been corrected for different parameters inside the model by linear regression and group size. The outcome, among corresponding p-values, had to be summed and recalculated for the groups with combined use of different graft materials in sinus augmentation surgery.

| Material | Subgroups (n) | Sinusses (n) |
|---|---|---|
| Autologous bone graft | 47 | 438 |
| PRP | 7 | 35 |
| MFDBA | 7 | 96 |
| ADBB | 29 | 319 |
| ADBB + autologous bone | 29 | 261 |
| ADBB + DFDBA | 9 | 113 |
| Synthetic HA | 10 | 108 |
| Phycogenic HA | 9 | 52 |
| β-TCP | 8 | 116 |
| β-TCP + autologous bone | 7 | 62 |
| Bioactive glass + autologous bone | 8 | 72 |
| TOTAL Σ | 170 | 1672 |

Table 2: Overview grafting materials. PRP, platelet-rich plasma; MFDBA, mineralized freeze-dried bone allograft; ADBB, anorganic deproteinized bovine bone; DFDBA, demineralized freeze-dried bone allograft; HA, hydroxyapatite; β-TCP, beta-tricalcium phosphate.

# RESULTS

The basis search provided 147 titles for consideration. As result 64 articles met our inclusion criteria. Describing autologous bone,[29;36;37;39;41;42;46-64], addition of PRP (Platelet Rich Plasma),[47;52;55] DFDBA (Demineralized Freeze Dried Bone Allograft),[65-69] MFDBA (Mineralized Freeze Dried Bone Allograft),[24;25;68;70-73] ADBB (Anorganic Deproteinized Bovine Bone),[24;26;48;53;60;62;69;74-85] BG (Bioactive Glass),[29;56;86;87] synthetic HA (Hydroxyapatite),[53;64;81;82;88-90] coral derived HA (Hydroxyapatite),[91;92] β-TCP (beta-tricalcium phosphate)[36;37;54;58-60;85;93-95] and combinations of these materials.[24;42;48;49;60;62;67;69;78;87;95-98] The majority of these 64 articles were prospective controlled studies (48). Only eight prospective randomized clinical trials met the inclusion criteria, followed by eight case series (Table 1). In total, histomorphometric data were obtained from 1677 grafted sinuses divided in 172 subgroups. As a result, 11 material groups were recognized of similar bone graft or combination of bone graft materials (Table 2). A specific overview of these subgroups is provided in Table 3-13. Graft materials were used as blocks, particulated grafts or as combination of both. The topic of using (non)-resorbable barrier membranes over the lateral wall was addressed in 24 out of the 64 articles.

**Statistical analysis**

In total 17 variables entered into the regression model as depicted in Table 14 along with their p-values and confidence intervals. The R-square for the full model was 0.460, the adjusted R-square 0.403.

***Graft material***

After linear regression a reference value for TBV of 63% was calculated. Most graft materials showed significant differences in TBV compared to this reference value (Table 14) (Figure 1). The addition of PRP to an autologous bone graft reduced the TBV with -18.0%. Usage of a xenogenic bone graft decreased the TBV with -13%, while usage of a xenogenic bone graft combined with autologous bone resulted in a less decrease of -8%. The confidence intervals showed a significant overlap, indicating that the addition of autologous bone made no statistically significant difference. Combining DFDBA with a xenogenic bone graft, however, resulted in a significant lower TBV, as a decrease of -25% compared to the reference was found. Combining BG with autologous bone resulted in a -17% decrease and combining β-TCP with autologous bone resulted in a reduction of -9% in TBV. Sinus floor augmentation with synthetically manufactured HA or hydrothermally converted coral reduced TBV with -11% or -12% respectively. Furthermore, almost significant, MFDBA and β-TCP resulted both in a TBV decrease of -7%. Thus, most bone substitutes, even mixed with autologous bone, resulted in a significant lower TBV compared to the refer-

ence value of 63% for autologous bone. On the other hand, taken into account that the confidence intervals of most substitutes had a significant overlap, the difference between them regarding amount of TBV was not statistically significant. This, however, did not account for DFDBA with a xenogenic bone graft.

***Biopsy time***

There is significant evidence that the TBV was influenced by graft healing time in general, thus the 'biopsy time' of all samples. Overall TBV was 8% or 6% higher if a biopsy was respectively taken before 4.5 months or after 9.0 months after the sinus augmentation surgery. After performing the second linear regression, (Table 15) correcting each bone graft material for 'biopsy time', a summation had to be made for the combined use of graft materials with their corresponding p-values (Table 16).

Only in case of autologous bone grafting and the combined use of autologous bone with a xenogenic bone graft, 'biopsy time' had a significant influence on the TBV: a biopsy time of less than 4.5 months resulted in an increase in TBV of 11% compared to a biopsy taken between 4.5 and 9.0 months. Additionally, a biopsy time of 9.0 months or longer increased the TBV with 10% compared to the centered group. Furthermore, the combined use of autologous bone and a xenogenic bone graft started with a plus of 26% of TBV compared to the period between 4.5 and 9 months. Surprisingly, no further significant difference could be detected between the various graft materials in time.

***Variables***

Compared to the reference value of TBV, usage of a particulated graft significantly decreased the TBV with -18%, while usage of a block resulted in an decrease of -6% TBV, although not to a significant level. In addition, 'delayed' implant placement, significantly resulted in a lower TBV of -7% compared to 'immediate' implant placement. Further, no evidence was found that the use of a resorbable membrane over the lateral window had any effect, positive or negative, on the amount of TBV.

| Author | Year | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|
| Lundgren | 1996 | Particulate | 10 | None | Delayed | 0 | 45.00 |
| Lundgren | 1996 | Block | 10 | None | Delayed | 0 | 58.00 |
| Pejrone | 2002 | Block | 26 | None | Delayed | 0 | 59.30 |
| Lorenzetti | 1998 | Block | 8 | None | Delayed | 0 | 60.00 |
| Lorenzetti | 1998 | Particulate | 3 | None | Delayed | 0 | 65.60 |
| Lorenzetti | 1998 | Block | 3 | None | Delayed | 0 | 65.60 |
| Thor | 2007 | Particulate | 11 | None | Delayed | 3 | 11.00 |
| Zerbo | 2003 | Block | 5 | Collagen | Delayed | 3 | 39.38 |
| Raghoebar | 2005 | Both | 5 | None | Delayed | 3 | 41.10 |
| Consolo | 2007 | Particulate | 2 | None | Delayed | 4 | 26.00 |
| Zerbo | 2003 | Block | 6 | Collagen | Delayed | 4 | 39.78 |
| Tadjoedin | 2000 | Particulate | 3 | None | Delayed | 4 | 40.94 |
| Lorenzetti | 1998 | Block | 3 | None | Delayed | 4 | 62.60 |
| Consolo | 2007 | Particulate | 2 | None | Delayed | 5 | 29.20 |
| Crespi | 2007 | Particulate | 6 | Collagen | Delayed | 5 | 34.00 |
| Tadjoedin | 2000 | Particulate | 3 | None | Delayed | 5 | 42.24 |
| John | 2004 | Particulate | 2 | None | Delayed | 5 | 53.50 |
| John | 2004 | Particulate | 2 | None | Immediate | 5 | 53.50 |
| Crespi | 2007 | Particulate | 10 | Collagen | Delayed | 5 | 69.70 |
| Barone | 2005 | Particulate | 18 | Collagen | Delayed | 5 | 70.00 |
| Crespi | 2009 | Particulate | 15 | None | Delayed | 5 | 78.40 |
| Gerressen | 2009 | Particulate | 15 | None | Delayed | 5 | 29.35 |
| Gerressen | 2009 | Particulate | 15 | None | Delayed | 5 | 37.87 |
| Thor | 2007 | Particulate | 11 | None | Delayed | 6 | 13.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Groeneveld | 1999 | Particulate | 3 | None | Delayed | 6 | 26.20 |
| van den Bergh | 2000 | Particulate | 3 | None | Delayed | 6 | 26.60 |
| Consolo | 2007 | Particulate | 2 | None | Delayed | 6 | 29.00 |
| Szabo | 2001 | Particulate | 4 | None | Delayed | 6 | 37.05 |
| Szabo | 2005 | Particulate | 20 | None | Delayed | 6 | 38.34 |
| Lundgren | 1996 | Particulate | 10 | None | Delayed | 6 | 40.00 |
| Scarano | 2006 | Particulate | 16 | None | Delayed | 6 | 40.10 |
| Zerbo | 2003 | Block | 3 | Collagen | Delayed | 6 | 40.90 |
| Zerbo | 2004 | Particulate | 5 | None | Delayed | 6 | 41.00 |
| Zijderveld | 2005 | Particulate | 6 | None | Delayed | 6 | 41.00 |
| Tadjoedin | 2000 | Particulate | 3 | None | Delayed | 6 | 43.65 |
| Lorenzetti | 1998 | Particulate | 8 | None | Delayed | 6 | 53.00 |
| Pejrone | 2002 | Block | 26 | None | Delayed | 6 | 54.10 |
| Suba | 2006 | Particulate | 17 | None | Delayed | 7 | 34.70 |
| Consolo | 2007 | Particulate | 2 | None | Delayed | 7 | 20.00 |
| Turunen | 2004 | Both | 17 | None | Delayed | 7 | 25.10 |
| Simunek | 2008 | Particulate | 8 | None | Delayed | 9 | 49.20 |
| Le Lorc'h-Bukiet | 2005 | Particulate | 24 | None | Delayed | 10 | 49.40 |
| Lorenzetti | 1998 | Particulate | 3 | None | Delayed | 11 | 69.30 |
| Turunen | 2004 | Both | 17 | None | Delayed | 12 | 25.10 |
| Lundgren | 1996 | Particulate | 10 | None | Delayed | 12 | 48.00 |
| Pejrone | 2002 | Block | 26 | None | Delayed | 12 | 63.90 |
| Hallman | 2002 | Particulate | 11 | None | Delayed | 13 | 37.30 |

Table 3: Overview autologous bone grafting

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Thor | 2007 | ABG + PRP | Particulate | 11 | None | Delayed | 3.0 | 22.00 |
| Raghoebar | 2005 | ABG + PRP | Both | 5 | None | Delayed | 3.0 | 38.40 |
| Consolo | 2007 | ABG + PRP | Particulate | 2 | None | Delayed | 4.0 | 43.30 |
| Consolo | 2007 | ABG + PRP | Particulate | 2 | None | Delayed | 5.0 | 39.30 |
| Thor | 2007 | ABG + PRP | Particulate | 11 | None | Delayed | 6.0 | 14.00 |
| Consolo | 2007 | ABG + PRP | Particulate | 2 | None | Delayed | 6.0 | 29.00 |
| Consolo | 2007 | ABG + PRP | Particulate | 2 | None | Delayed | 7.0 | 20.00 |

Table 4: Overview addition of PRP

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Choukroun | 2006 | FDBA | Particulate | 3 | None | Delayed | 4.0 | 20.31 |
| Stacchi | 2008 | FFB | Particulate | 10 | Collagen | Delayed | 5.0 | 48.15 |
| Kassolis | 2005 | FDBA | Particulate | 10 | Collagen | Delayed | 5.3 | 26.50 |
| Froum | 2006 | Puros | Particulate | 13 | Collagen | Delayed | 6.6 | 28.25 |
| Kolerman | 2008 | FDBA | Particulate | 23 | Collagen | Delayed | 9.0 | 29.09 |
| Noumbissi | 2005 | Puros | Particulate | 6 | None | Delayed | 9.0 | 40.33 |
| Cammack | 2005 | FDBA | Particulate | 31 | (non)- resorbable | Delayed | 11.2 | 41.07 |

Table 5: Overview MFDBA

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Wheeler | 1996 | Interpore 200 | Particulate | 4 | None | Delayed | 4.00 | 12.02 |
| Orsini | 2006 | Pig bone | Particulate | 10 | Collagen | Delayed | 5.00 | 36.00 |
| John | 2004 | Bio Oss | Particulate | 7 | None | Delayed | 5.50 | 29.52 |
| John | 2004 | Bio Oss | Particulate | 14 | None | Immediate | 5.50 | 29.52 |
| Yildirim | 2000 | Bio Oss | Particulate | 3 | Collagen | Delayed | 6.00 | 13.15 |
| Lee | 2006 | Bio Oss | Particulate | 14 | Collagen | Delayed | 6.00 | 18.30 |
| Valentini | 2000 | Bio Oss | Particulate | 20 | None | Delayed | 6.00 | 21.08 |
| Mangano | 2007 | Bio Oss | Particulate | 20 | None | Immediate | 6.00 | 36.20 |
| Scarano | 2006 | PepGen P-15 | Particulate | 16 | None | Delayed | 6.00 | 37.00 |
| Scarano | 2006 | Bio Oss | Particulate | 16 | None | Delayed | 6.00 | 39.00 |
| Yildirim | 2000 | Bio Oss | Particulate | 2 | Collagen | Delayed | 6.50 | 19.15 |
| Froum | 2006 | Bio Oss | Particulate | 13 | Collagen | Delayed | 6.60 | 12.44 |
| Cordaro | 2008 | Bio Oss | Particulate | 23 | Collagen | Delayed | 6.70 | 19.80 |
| Yildirim | 2000 | Bio Oss | Particulate | 2 | Collagen | Delayed | 7.00 | 10.85 |
| Ozyuvaci | 2003 | Bio Oss | Particulate | 20 | None | Immediate | 7.00 | 47.50 |
| Froum | 2008 | Bio Oss | Particulate | 11 | Collagen | Delayed | 7.17 | 22.30 |
| Froum | 1998 | Osteograft/n | Particulate | 5 | None | Delayed | 7.50 | 8.50 |
| Yildirim | 2000 | Bio Oss | Particulate | 2 | Collagen | Delayed | 7.50 | 15.25 |
| Froum | 1998 | Osteograft/n | Particulate | 10 | None | Delayed | 7.50 | 17.00 |
| Springer | 2006 | Bio Oss | Particulate | 5 | None | Delayed | 8.00 | 25.00 |
| Yildirim | 2000 | Bio Oss | Particulate | 2 | Collagen | Delayed | 9.00 | 16.50 |
| Simunek | 2008 | Bio Oss | Particulate | 10 | None | Delayed | 9.00 | 34.20 |
| Wheeler | 1996 | Interpore 200 | Particulate | 2 | None | Delayed | 10.00 | 25.10 |

Table 6/1: Overview ADBB grafting

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Lee | 2006 | Bio Oss | Particulate | 14 | Collagen | Delayed | 12.00 | 26.60 |
| Valentini | 2000 | Bio Oss | Particulate | 20 | None | Delayed | 12.00 | 27.55 |
| Artzi | 2002 | Bio Oss | Block | 10 | Collagen | Immediate | 12.00 | 34.20 |
| Artzi | 2001 | Bio Oss | Particulate | 4 | Collagen | Delayed | 12.00 | 42.10 |
| Artzi | 2001 | Bio Oss | Particulate | 16 | Collagen | Immediate | 12.00 | 42.10 |
| Hallman | 2002 | Bio Oss | Particulate | 14 | Collagen | Delayed | 14.50 | 41.70 |
| Traini | 2008 | Bio Oss | Particulate | 10 | None | Delayed | 20.00 | 38.00 |

Table 6/2: Overview ADBB grafting

| Author | Year | Graft name (ratio) | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Tadjoedin | 2003 | Bio Oss + ABG 1:5 | Particulate | 2 | None | Delayed | 5.0 | 37.30 |
| Barone | 2005 | Osteobiol + ABG 1:1 | Particulate | 18 | Collagen | Delayed | 5.0 | 67.00 |
| John | 2004 | Bio Oss + ABG 66:33 | Particulate | 7 | None | Delayed | 5.5 | 32.23 |
| John | 2004 | Bio Oss + ABG 66:33 | Particulate | 6 | None | Immediate | 5.5 | 32.23 |
| Wheeler | 1996 | Interpore 200 + ABG | Particulate | 2 | None | Delayed | 6.0 | 4.72 |
| Yildirim | 2001 | Bio Oss + ABG | Particulate | 2 | Collagen | Delayed | 6.0 | 15.17 |
| Yildirim | 2001 | Bio Oss + ABG | Particulate | 2 | Collagen | Delayed | 6.0 | 15.67 |
| Wheeler | 1996 | Interpore 200 + ABG | Particulate | 2 | None | Delayed | 6.0 | 23.00 |
| Galindo | 2008 | Bio Oss + ABG 1:1 | Particulate | 5 | Collagen | Delayed | 6.0 | 31.02 |
| Yildirim | 2001 | Bio Oss + ABG | Particulate | 2 | Collagen | Delayed | 6.5 | 18.27 |
| Yildirim | 2001 | Bio Oss + ABG | Particulate | 2 | Collagen | Delayed | 6.5 | 20.62 |
| Hallman | 2001 | Bio Oss + ABG 18:82 | Particulate | 20 | None | Delayed | 6.7 | 31.40 |
| Hallman | 2001 | Bio Oss + ABG 18:82 | Particulate | 20 | None | Delayed | 6.7 | 31.40 |
| Wheeler | 1996 | Interpore 200 + ABG | Particulate | 3 | None | Delayed | 7.0 | 14.82 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Wheeler | 1996 | Interpore 200 + ABG | Particulate | 2 | None | Delayed | 7.0 | 15.60 |
| Froum | 1998 | Osteograft/n + ABG | Particulate | 7 | None | Delayed | 7.5 | 18.50 |
| Froum | 1998 | Osteograft/n + ABG | Particulate | 31 | None | Delayed | 7.5 | 29.00 |
| Wallace | 2005 | Bio Oss + ABG 5:1 | Particulate | 6 | None | Delayed | 8.0 | 12.10 |
| Wallace | 2005 | Bio Oss + ABG 5:1 | Particulate | 21 | e-PTFE | Delayed | 8.0 | 16.90 |
| Wallace | 2005 | Bio Oss + ABG 5:1 | Particulate | 37 | Collagen | Delayed | 8.0 | 17.60 |
| Moy | 1993 | Interpore 200 + ABG 1:1 | Both | 4 | None | Delayed | 8.0 | 44.40 |
| Wheeler | 1996 | Interpore 200 + ABG | Particulate | 2 | None | Delayed | 9.0 | 12.60 |
| Yildirim | 2001 | Bio Oss + ABG | Particulate | 2 | Collagen | Delayed | 9.0 | 18.88 |
| Simunek | 2008 | Bio Oss + ABG 85:15 | Particulate | 10 | None | Delayed | 9.0 | 24.40 |
| Lorenzetti | 1998 | Interpore 200 + ABG 1:1 | Particulate | 3 | None | Delayed | 12.0 | 43.60 |
| Artzi | 2005 | Bio Oss + ABG | Particulate | 2 | Collagen | Delayed | 12.0 | 45.60 |
| Artzi | 2005 | Bio Oss + ABG | Particulate | 10 | Collagen | Immediate | 12.0 | 45.60 |
| Hallman | 2002 | ABG + Bio Oss 1:4 | Particulate | 11 | None | Delayed | 12.5 | 39.90 |
| Hallman | 2001 | ABG + Bio Oss 18:82 | Particulate | 20 | None | Delayed | 36.0 | 51.50 |

Table 7: Overview ADBB grafting and autologous bone

| Author | Year | Graft name (ratio) | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Hanisch | 1999 | Osteograft/n + DFDBA 1:1 | Particulate | 20 | None | Delayed | 6.0 | 8.10 |
| Froum | 1998 | Osteograft/n + DFDBA | Particulate | 8 | None | Delayed | 7.5 | 14.00 |
| Froum | 1998 | Osteograft/n + DFDBA | Particulate | 14 | None | Delayed | 7.5 | 23.00 |
| Moy | 1993 | Interpore + DBP 200 7:1 | Both | 2 | None | Delayed | 8.0 | 4.60 |
| Hanisch | 1999 | Osteograft/n + DFDBA 1:1 | Particulate | 20 | None | Delayed | 8.0 | 9.00 |
| Noumbissi | 2005 | Bio Oss + DFDBA 1:1 | Particulate | 4 | None | Delayed | 9.0 | 38.75 |
| Hanisch | 1999 | Osteograft/n + DFDBA 1:1 | Particulate | 20 | None | Delayed | 10.0 | 11.80 |
| Landi | 2000 | Osteograft/n + DFDBA 1:1 | Particulate | 5 | None | Delayed | 10.3 | 27.92 |
| Hanisch | 1999 | Osteograft/n + DFDBA 1:1 | Particulate | 20 | None | Delayed | 12.0 | 20.70 |

Table 8: Overview ADBB and DFDBA graft

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Canullo | 2009 | Nanobone | Particulate | 8 | None | Delayed | 3.0 | 8.00 |
| Crespi | 2009 | SINTlife | Particulate | 15 | None | Delayed | 5.0 | 29.65 |
| Scarano | 2006 | Fin granule HA | Particulate | 16 | None | Delayed | 6.0 | 32.00 |
| Mangano | 2007 | Porous synthetic HA | Particulate | 20 | None | Immediate | 6.0 | 34.70 |
| Mangano | 2006 | Engipore | Both | 11 | None | Delayed | 6.0 | 38.50 |
| Canullo | 2009 | Nanobone | Particulate | 8 | None | Delayed | 6.0 | 48.00 |
| Artzi | 2003 | Osteogen | Particulate | 2 | Collagen | Delayed | 12.0 | 28.10 |
| Artzi | 2003 | Osteogen | Particulate | 8 | Collagen | Immediate | 12.0 | 28.10 |
| Artzi | 2001 | Osteogen | Particulate | 4 | Collagen | Delayed | 12.0 | 32.20 |
| Artzi | 2001 | Osteogen | Particulate | 16 | Collagen | Immediate | 12.0 | 32.20 |

Table 9: Synthetic HA

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Simunek | 2005 | Algipore | Particulate | 3 | None | Delayed | 6.0 | 10.90 |
| Simunek | 2005 | Algipore | Particulate | 3 | None | Immediate | 6.0 | 20.10 |
| Ewers | 2005 | Algipore | Particulate | 29 | various | Delayed | 7.1 | 28.95 |
| Simunek | 2005 | Algipore | Particulate | 3 | None | Delayed | 9.0 | 25.00 |
| Simunek | 2005 | Algipore | Particulate | 3 | None | Immediate | 9.0 | 31.70 |
| Simunek | 2005 | Algipore | Particulate | 2 | None | Delayed | 12.0 | 33.50 |
| Simunek | 2005 | Algipore | Particulate | 3 | None | Immediate | 12.0 | 34.80 |
| Simunek | 2005 | Algipore | Particulate | 3 | None | Delayed | 15.0 | 30.20 |
| Simunek | 2005 | Algipore | Particulate | 3 | None | Immediate | 15.0 | 51.10 |

Table 10: Overview coral derived HA

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Zerbo | 2004 | Cerasorb | Particulate | 9 | None | Delayed | 6.0 | 17.00 |
| Zijderveld | 2005 | Cerasorb | Particulate | 10 | None | Delayed | 6.0 | 17.00 |
| Szabo | 2001 | Cerasorb | Particulate | 8 | None | Delayed | 6.0 | 29.37 |
| Szabo | 2005 | Cerasorb | Particulate | 40 | None | Delayed | 6.0 | 36.47 |
| Suba | 2006 | Cerasorb | Particulate | 17 | None | Delayed | 6.5 | 32.38 |
| Ozyuvaci | 2003 | β-TCP | Particulate | 20 | None | Immediate | 7.0 | 52.50 |
| Zerbo | 2001 | Cerasorb | Particulate | 2 | None | Delayed | 8.0 | 20.00 |
| Simunek | 2008 | Cerasorb | Particulate | 10 | None | Delayed | 9.0 | 21.40 |

Table 11: Overview β-TCP

| Author | Year | Graft name | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Knabe | 2008 | β-TCP + ABG | Particulate | 10 | None | Delayed | 6.0 | 26.70 |
| Knabe | 2008 | β-TCP + ABG | Particulate | 10 | None | Delayed | 6.0 | 31.70 |
| Knabe | 2008 | β-TCP + ABG | Particulate | 10 | None | Delayed | 6.0 | 35.50 |
| Knabe | 2008 | β-TCP + ABG | Particulate | 10 | None | Delayed | 6.0 | 40.30 |
| Simunek | 2008 | Cerasorb + ABG | Particulate | 10 | None | Delayed | 9.0 | 24.00 |
| Artzi | 2005 | β-TCP + ABG | Particulate | 3 | Collagen | Delayed | 12.0 | 32.00 |
| Artzi | 2005 | β-TCP + ABG | Particulate | 9 | Collagen | Immediate | 12.0 | 32.00 |

Table 12: Overview β-TCP and autologous bone

| Author | Year | Graft name (ratio) | Particulated or Block | N (sinusses) | Membrane | Immediate or Delayed implant placement | Biopsy time (months) | TBV (%) |
|---|---|---|---|---|---|---|---|---|
| Tadjoedin | 2000 | BG + ABG 1:1 | Particulate | 3 | None | Delayed | 4.0 | 28.45 |
| Tadjoedin | 2000 | BG + ABG 1:1 | Particulate | 3 | None | Delayed | 5.0 | 34.54 |
| Galindo | 2008 | BG + ABG 1:1 | Particulate | 5 | Collagen | Delayed | 6.0 | 33.08 |
| Tadjoedin | 2000 | BG + ABG 1:1 | Particulate | 3 | None | Delayed | 6.0 | 38.07 |
| Turunen | 2004 | BG S53P4 + ABG | Particulate | 17 | None | Delayed | 7.0 | 25.70 |
| Cordioli | 2001 | BG + ABG 4:1 | Particulate | 12 | Collagen | Immediate | 10.8 | 14.20 |
| Cordioli | 2001 | BG + ABG 4:1 | Particulate | 12 | Collagen | Immediate | 10.8 | 30.60 |
| Turunen | 2004 | BG S53P4 + ABG | Particulate | 17 | None | Delayed | 12.0 | 28.80 |

Table 13: Overview bioactive glass

| Model | | Unstandardized Coefficients | | Sig. | 95% Confidence Interval for B | |
|---|---|---|---|---|---|---|
| | | B | Std. Error | *p* | Lower Bound | Upper Bound |
| 1 | (Constant) | 62.66 | 8.28 | 0.00 | 52.77 | 85.48 |
| | Particulated graft | -17.89 | 5.64 | 0.00 | -29.02 | -6.76 |
| | Block graft | -5.85 | 4.79 | 0.22 | -15.31 | 3.62 |
| | Membrane | -13.14 | 8.05 | 0.10 | -29.04 | 2.75 |
| | Resorbable membrane | 11.47 | 8.18 | 0.16 | -4.68 | 27.62 |
| | Delayed implantology | -6.46 | 2.91 | 0.03 | -12.20 | -0.72 |
| | Biopsy t<4.5 months | 8.41 | 2.56 | 0.00 | 3.35 | 13.47 |
| | Biopsy t>9.0 months | 5.57 | 2.13 | 0.01 | 1.35 | 9.78 |
| | PRP | -17.96 | 6.27 | 0.01 | -30.33 | -5.59 |
| | MFDBA | -7.30 | 4.52 | 0.11 | -16.23 | 1.63 |
| | ADBB | -12.74 | 3.02 | 0.00 | -18.71 | -6.77 |
| | ADBB + Autologous | -8.26 | 3.13 | 0.01 | -14.44 | -2.09 |
| | ADBB + DFDBA | -25.31 | 3.97 | 0.00 | -33.16 | -17.47 |
| | Synthetic HA | -11.30 | 4.10 | 0.01 | -19.40 | -3.20 |
| | Coral derived HA | -11.50 | 5.48 | 0.04 | -22.32 | -0.67 |
| | β-TCP | -6.53 | 4.04 | 0.11 | -14.50 | 1.45 |
| | β-TCP + autologous | -9.19 | 5.00 | 0.07 | -19.06 | 0.69 |
| | Bioactive glass + autologous | -16.82 | 4.79 | 0.00 | -26.28 | -7.35 |

Table 14: Regression analysis 1. Dependent Variable: TBV.
(Example: Calculation of expected TBV for: "Particulated synthetic HA with a resorbable membrane and Immediate implantology and a biopsy time of <4.5 months" = 62.66 – (17.89 – 11.3 + 11.47 + 8.41) = 53.35% ).

| Model | | Unstandardized Coefficients | | Sig. *p* | 95% Confidence Interval for B | |
|---|---|---|---|---|---|---|
| | | B | Std. Error | | Lower Bound | Upper Bound |
| 2 | (Constant) | 62.20 | 8.41 | 0.00 | 52.29 | 85.52 |
| | Particulated graft | -18.91 | 5.54 | 0.00 | -29.86 | -7.95 |
| | Block graft | -7.52 | 4.73 | 0.11 | -16.87 | 1.82 |
| | Membrane | -8.16 | 7.96 | 0.31 | -23.90 | 7.57 |
| | Resorbable membrane | 7.31 | 8.08 | 0.37 | -8.65 | 23.27 |
| | Delayed implantology | -6.70 | 2.99 | 0.03 | -12.60 | -0.80 |
| | PRP t<4.5 months | 4.18 | 12.28 | 0.73 | -20.09 | 28.46 |
| | PRP t=4.5-9.0 months | -19.79 | 9.22 | 0.03 | -38.01 | -1.57 |
| | Autologous t<4.5 months | 10.86 | 3.79 | 0.00 | 3.37 | 18.34 |
| | Autologous t>9 months | 9.52 | 4.31 | 0.03 | 0.99 | 18.05 |
| | MFDBA t<4.5 months | 6.74 | 11.78 | 0.57 | -16.55 | 30.03 |
| | MFDBA t=4.5-9.0 months | -7.49 | 10.11 | 0.46 | -27.48 | 12.50 |
| | MFDBA t>9 months | 8.07 | 10.39 | 0.44 | -12.46 | 28.60 |
| | ADBB t<4.5 months | 1.17 | 6.29 | 0.85 | -11.27 | 13.61 |
| | ADBB t=4.5-9.0 months | -10.82 | 3.96 | 0.01 | -18.65 | -3.00 |
| | ADBB t>9 months | 6.19 | 4.24 | 0.15 | -2.19 | 14.57 |
| | ADBB + autologous t<4.5 months | 14.83 | 7.55 | 0.05 | -0.10 | 29.76 |
| | ADBB + autologous t=4.5-9.0 months | -11.53 | 4.06 | 0.01 | -19.54 | -3.51 |
| | ADBB + autologous t>9 months | 5.40 | 6.79 | 0.43 | -8.03 | 18.82 |
| | ADBB + DFDBA t=4.5-9.0 months | -24.09 | 5.10 | 0.00 | -34.17 | -14.00 |
| | ADBB + DFDBA t>9 months | 6.77 | 6.45 | 0.30 | -5.97 | 19.51 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Synthetic HA t<4.5 months | -13.56 | 8.56 | 0.12 | -30.47 | 3.36 |
| | Synthetic HA t=4.5-9.0 months | -0.91 | 5.49 | 0.87 | -11.78 | 9.95 |
| | Synthetic HA t>9 months | -9.35 | 8.20 | 0.26 | -25.55 | 6.85 |
| | Coral derived HA t=4.5-9.0 months | -9.81 | 6.70 | 0.15 | -23.05 | 3.42 |
| | Coral derived HA t>9 months | 4.11 | 10.33 | 0.69 | -16.30 | 24.52 |
| | β-TCP t=4.5-9.0 months | -3.35 | 4.40 | 0.45 | -12.06 | 5.35 |
| | β-TCP t>9 months | -11.84 | 11.24 | 0.29 | -34.06 | 10.39 |
| | β-TCP + autologous t=4.5-9.0 months | -3.04 | 6.08 | 0.62 | -15.06 | 8.98 |
| | β-TCP + autologous t>9 months | -16.98 | 10.13 | 0.10 | -37.01 | 3.05 |
| | Bioactive glass + autologous t<4.5 months | -8.19 | 15.91 | 0.61 | -39.65 | 23.26 |
| | Bioactive glass + autologous t=4.5-9.0 months | -7.76 | 7.38 | 0.29 | -22.34 | 6.83 |
| | Bioactive glass + autologous t>9 months | -16.72 | 9.82 | 0.09 | -36.13 | 2.69 |

Table 15: Regression analysis 2. (Reference group is Autologous bone grafting and biopsy time between t =4.5-9.0 months)

| Augmentation Material | | t<4.5 compared to t=4.5-9.0 | | | t>9.0 compared to t=4.5-9.0 | |
|---|---|---|---|---|---|---|
| | Effect | 95% CI | *p*-value | Effect | 95% CI | p-value |
| Autologous | 10.86% | [2.41, 19.3] | 0.02 | 9.52% | [-0.1, 19.14] | 0.05 |
| PRP | 15.04% | [-13.62, 43.71] | 0.27 | | | |
| MFDBA | 6.74% | [-19.53, 33.02] | 0.58 | 8.07% | [-15.1, 31.23] | 0.46 |
| ADBB | 1.17% | [-12.87, 15.2] | 0.86 | 6.19% | [-3.27, 15.65] | 0.18 |
| ADBB + Autologous | 25.69% | [6.84, 44.53] | 0.01 | 5.40% | [-9.75, 20.54] | 0.45 |
| ADBB + DFDBA | | | | 6.77% | [-7.6, 21.15] | 0.32 |
| Synthetic HA | -13.56% | [-32.64, 5.53] | 0.14 | -9.35% | [-27.63, 8.93] | 0.28 |
| Phycogenic HA | | | | 4.11% | [-18.92, 27.14] | 0.70 |
| β-TCP | | | | -11.84% | [-36.91, 13.24] | 0.32 |
| β-TCP + Autologous | | | | -16.98% | [-39.58, 5.62] | 0.12 |
| Bioactive glass + Autologous | | | | -7.20% | [-31.12, 16.72] | 0.52 |

Table 16: Summation of regression analysis 2

# DISCUSSION

Maxillary sinus floor augmentations are ideal test sites to histomorphometrically assess a grafted material. Before preparing the implant bed to install dental implants, a biopsy of the reconstructed area can be easily taken, implicating no extra burden for the patient. As an additional advantage this procedure can be performed under local anesthesia. In the selected studies various bone substitutes were used, or solely or as a bone graft extender in combination with autologous bone. Till now, autologous bone grafts are considered to be the gold standard.[8;9] This postulation, however, is only based on implant survival, while bone quality in the grafted area is often left out of consideration.[7;27] Furthermore, implant survival and bone quality may be confounded by factors other than the graft material.[7;38] The aim of this study was to give a powerful estimate of the true effect of the various variables: 'graft material', 'biopsy time', 'block grafting technique', 'particulated grafting technique', the usage of a '(non)-resorbable membrane' over the lateral window, and 'immediate or delayed' implant placement, on the histomorphometric outcome after sinus floor augmentation surgery. Because of the general absence or differences of other histomorphometric indices in the studies, TBV was solely used as dependent variable.

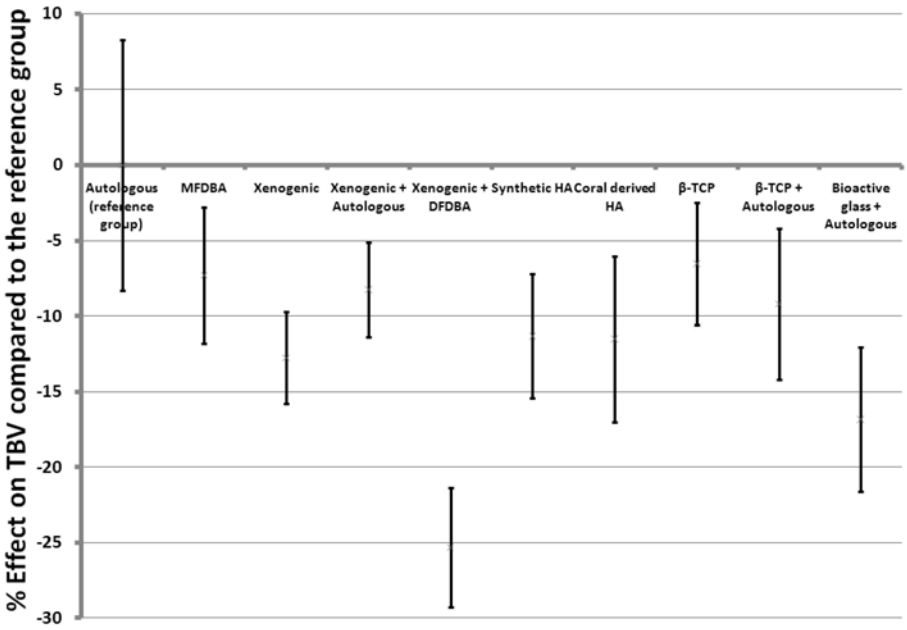


Figure 1. Overview of the effect of type of grafting material on total bone volume compared to the reference (Table 14).

### Graft material

#### *Autologous bone*

Compared to autologous bone, for each biomaterial or combination of graft materials in sinus augmentation surgery a significant lower TBV was found. Evidently, autologous bone grafting resulted in the highest percentage of mineralized bone. It should, however, be emphasized that when evaluating biopsies from autologous bone grafted areas, not only the new bone formation, but also the transplanted bone volume is scored. This in contrast to examining biopsies from sites reconstructed with bone substitutes, from which only the new formed bone can be measured.

#### *PRP*

Platelets are a natural source of growth factors. Some authors state that the combined use of growth factors and graft material will introduce osteogenesis and improve bone healing,[99] while others reject the adjunctive use Platelet-Rich Plasma (PRP) in sinus augmentation because of disappointing results.[100;101] In this study, the addition of PRP to a autologous bone graft generally resulted in a significant lower TBV. In the literature, the regenerative potential of PRP seemed to be restricted to shorter treatment times.[47] However, in this meta-analysis, no significance evidence was found that PRP has a positive effect on TBV during graft healing time. To date, none of the studies, describing the use of growth factors, for example BMP-2, BMP-7 or TGF-β, fulfilled the inclusion criteria.

#### *Allogenic bone*

DFDBA was always used in combination with a xenogenic bone graft and resulted in the lowest TBV as compared to autologous bone and all other materials. Grafting with MFDBA has a tendency to result in a slightly lower TBV compared to autologous bone, but not to a significant level. Also in case of MFDBA, it must be noted that particles of non-resorbed MFDBA are described to be difficult to distinguish as graft material from new vital bone in the calculation of TBV.[24]

#### *Xenogenetic bone*

The addition of autologous bone to a xenograft resulted in a slight increase in TBV compared to its single use, but not to a significant level. This increase in TBV varied between 15% till 50%.[42;60] As the ratio of xenogenic bone mineral versus autologous bone graft increases, resorption of the bone additive decreases exponentially, because less osteoclasts can be recruited from the autogenous bone.[27;102] A reduced resorption may have negative consequences on the mechanical properties of the augmented bone and its capacity to support an implant, since the augmented bone will be a composite rather than a homogenous bone structure.[27;103] Obviously, this is the case for all bone substitutes.

***Alloplastic bone substitutes***

A variety of alloplastic bone substitutes, single or in combination with autologous bone, was used in sinus augmentation surgery. In this study the effect of BG, synthetic HA, coral derived HA and β-TCP on the amount of TBV was investigated. Although alternative materials were described in the literature, they did not met the inclusion criteria stated for this meta-analysis. To add the osteogenic and osteoinductive components that are necessary to achieve complete bone formation, the bone substitutes were occasionally mixed with autogenous bone.[24;42;48;49;60;62;67;69;78;87;95-98] Furthermore, in larger defects the bone additive reduces the required autologous bone needed.

BG was used in combination with autologous bone in ratios of 1:1 and 1:4. BG is a resorbable particulate synthetic bioactive glass from which the granules are supposed to function as small bone regenerative chambers.[104] Unexpectedly, after linear regression, sinus augmentation with BG, resulted in the lowest TBV of all alloplastic materials. Sinus augmentation with synthetic or coral derived HA also resulted in a decrease of TBV. As HA was grafted without the addition of autologous bone, TBV was only influenced by new bone formation from the local sinus environment. For β-TCP with or without the addition of autologous bone, TBV differed not significantly. While others stated that, along with the replacement of solely β-TCP, the TBV will consequently increase,[58] this postulation, however, could not be confirmed by this meta-analytical study. Also the influence of adding autologous bone to β-TCP appeared to be negligible, although supplemented in 10% to 50% of the total graft volume.[60;93;95]

All bone graft substitutes, alone or in combination with an autologous bone graft, resulted in a analogous significant lower TBV compared to autologous bone grafting. On the other hand, taken into account that the confidence intervals of most substitutes had a significant overlap, the differences between them regarding amount of TBV were not significant.

In a recent review by Nkenke et al., the current literature was analyzed in order to determine whether there are advantages of using autogenous bone over bone substitutes in sinus floor augmentation with respect to implant survival. They concluded that no evidence was present that neither supports nor refutes the superiority of autologous bone grafts over other graft materials with regard to implant survival.[7] In our study, there is a significant difference between autologous bone and their alternatives with respect to the TBV. However, the higher TBV apparently does not result in a higher implant survival.[7] Therefore, when using bone substitutes, it is still unclear what the minimal TBV is for a grafted sinus to guarantee implant survival.

**Biopsy time**

In literature it is reported that up to 33% of the autologous bone graft may resorb during the initial six months after sinus floor augmentation surgery.[47;51;105;106] This decrease in TBV affects the primary implant stability and therefore, as this effect of significant initial bone resorption may persist for years,[107-109] is a serious problem. In this meta-analysis, autologous graft resorption resulted in a significant lower TBV between 4.5 and 9.0 months. Hereafter, the TBV raised to same level of TBV, as scored in the first 4.5 months.

For the combination ADBB and autologous bone, biopsies taken in the first 4.5 months after initial surgery resulted in a significant higher TBV compared to biopsies taken at a later time point. Surprisingly, addition of autologous bone to the other bone substitutes did not result into this boost effect; the TBV did not significantly alter in time. Recently, Nkenke et al. concluded in a review that implant survival seemed not to be influenced by the healing period of the graft material.[7] This is in analogue with our finding that in case of using bone substitutes, the TBV is constant in time. Because of the wide variation and absence of other (cellular) histomorphometric indices in studies, no further conclusion could be drawn about resorption, bone apposition of remodeling in time in our study.

**Variables**

After statistical analysis, particulation of the graft resulted in a significant lower amount of TBV, but there was no such evidence for block grafting. Almost all grafted materials were used in a particulated structure. Occasionally, autologous bone was used as block graft, but only a few articles compared block versus particulate grafting.[26;39;49-52;56;67;88]

Placement of endosseous dental implants is done either simultaneously or after a certain time period to allow for consolidation of the grafted material. Simultaneous implant placement is less invasive and more effective.[110] Also, 'delayed' implant placement resulted in a significant decrease of TBV compared to 'immediate' implant placement. However, residual alveolar ridge height and implant stability should be the decisive argument for the decision of staged implant placement.[111]

Another examined variable was the use of a membrane over the lateral window of the sinus. Tarnow et al. reported that the placement of a e-PTFE (expanded polytetrafluoroethylene) barrier membrane tends to increase vital bone formation.[112] Others suggested that this effect also can be achieved using a poly (lactic acid) membrane.[113] In a meta-analysis on the survival of endosseous dental implants, Wallace et al., concluded that membrane utilization is a useful adjunctive therapy that results in an increased survival rate of implant in the

grafted maxillary sinus.[38] This increase in implant survival could be explained by a higher percentage of bone volume.[38;112] However, our study shows no significant effect, positive or negative, of the use of a (non)-resorbable membrane over the lateral window on the amount of TBV.

## CONCLUSION

In conclusion, 'particulate grafting' 'immediate and delayed implant placement,' and 'biopsy time' were determined as general significant variables on the histomorphometric outcome of TBV after sinus floor augmentation surgery using various biomaterials. Allogenic, xenogenic and alloplastic graft materials or combinations will result in a significant lower TBV compared to autologous bone grafting. The addition of PRP to an autologous bone graft in sinus augmentation has a negative effect on the TBV. In the second analysis, inventorying the effect of 'biopsy time' for autologous bone, the TBV was significantly higher before 4.5 and after 9.0 months of healing time compared to period in between. For bone substitutes only ADBB in combination with autologous bone performed significant higher in the first 4.5 months. Surprisingly, for all other bone substitutes no significant effect on TBV in time could be proven. Based on this histomorphometric meta-analysis autologous bone grafting results in the highest TBV and has still to be considered to be the gold standard. All described bone graft substitutes showed less TBV. However, it must be emphasized that the consequence of the TBV for implant survival is still unraveled yet.

# REFERENCES


1. Kaufman E. Maxillary sinus elevation surgery: an overview. Journal of Esthetic and Restorative Dentistry 15, 272, 2003.
2. Boyne P.J. and James R.A. Grafting of the maxillary sinus floor with autogenous marrow and bone. Journal of Oral Surgery 38, 613, 1980.
3. Misch C.E. Maxillary sinus augmentation for endosteal implants: organized alternative treatment plans. International Journal Oral Implantology 4, 49, 1987.
4. Esposito M., Grusovin M.G., Worthington H.V., and Coulthard P. Interventions for replacing missing teeth: bone augmentation techniques for dental implant treatment. Cochrane Database Syst Rev 17,3, CD003607, 2006.
5. Esposito M., Grusovin M.G., Coulthard P., and Worthington H.V. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. International Journal of Oral & Maxillofacial Implants 21, 696, 2006.
6. Del Fabbro M., Testori T., Francetti L., and Weinstein R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. International Journal of Periodontics & Restorative Dentistry 24, 565, 2004.
7. Nkenke E. and Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clinical Oral Implants Research 20 Suppl 4, 124, 2009.
8. Tong D.C., Rioux K., Drangsholt M., and Beirne O.R. A review of survival rates for implants placed in grafted maxillary sinuses using meta-analysis. International Journal of Oral & Maxillofacial Implants 13, 175, 1998.
9. Jensen O.T., Shulman L.B., Block M.S., and Lacono V.J. Report of the Sinus Consensus Conference of 1996. International Journal of Oral & Maxillofacial Implants 13 Suppl, 11, 1998.
10. Cohen M., Figueroa A.A., Haviv Y., Schafer M.E., and Aduss H. Iliac versus cranial bone for secondary grafting of residual alveolar clefts. Plastic and Reconstructive Surgery 87, 423, 1991.
11. Eppley B.L. Donor site morbidity of rib graft harvesting in primary alveolar cleft bone grafting. Journal of Craniofacial Surgery 16, 335, 2005.
12. Hoppenreijs T.J., Nijdam E.S., and Freihofer H.P. The chin as a donor site in early secondary osteoplasty: a retrospective clinical and radiological evaluation. Journal of Cranio-Maxillofacial Surgery 20, 119, 1992.
13. Sindet-Pedersen S. and Enemark H. Reconstruction of alveolar clefts with mandibular or iliac crest bone grafts: a comparative study. Journal of Oral and Maxillofacial Surgery 48, 554, 1990.
14. Swan M.C. and Goodacre T.E. Morbidity at the iliac crest donor site following bone grafting of the cleft alveolus. British Journal of Oral and Maxillofacial Surgery 44, 129, 2006.
15. Damien C.J. and Parsons J.R. Bone graft and bone graft substitutes: a review of current technology and applications. Journal of Applied Biomaterials 2, 187, 1991.
16. Canady J.W., Zeitler D.P., Thompson S.A., and Nicholas C.D. Suitability of the iliac crest as a site for harvest of autogenous bone grafts. Cleft Palate Craniofacial Journal 30, 579, 1993.
17. Beirne J.C, Barry H.J., Brady F.A., and Morris V.B. Donor site morbidity of the anterior iliac crest following cancellous bone harvest. International Journal of Oral and Maxillofacial Surgery 25, 268, 1996.
18. Bahr W. and Coulon J.P. Limits of the mandibular symphysis as a donor site for bone grafts in early secondary cleft palate osteoplasty. International Journal of Oral and Maxillofacial Surgery 25, 389, 1996.
19. Borstlap W.A., Heidbuchel K.L., Freihofer H.P., and Kuijpers-Jagtman A.M. Early secondary bone grafting of alveolar cleft defects. A comparison between chin and rib grafts. Journal of Cranio-Maxillofacial Surgery 18, 201, 1990.
20. Koole R., Bosker H., and van der Dussen F.N. Late secondary autogenous bone grafting in cleft patients comparing mandibular (ectomesenchymal) and iliac crest (mesenchymal) grafts. Journal of Cranio-Maxillofacial Surgery 17 Suppl 1, 28, 1989.

21. Raghoebar G.M., Meijndert L., Kalk W.W., and Vissink A. Morbidity of mandibular bone harvesting: a comparative study. International Journal of Oral & Maxillofacial Implants 22, 359, 2007.
22. Nwoku A.L., Al Atel A., Al Shlash S., Oluyadi B.A., and Ismail S. Retrospective analysis of secondary alveolar cleft grafts using iliac of chin bone. Journal of Craniofacial Surgery 16, 864, 2005.
23. Wheeler S.L. Sinus augmentation for dental implants: the use of alloplastic materials. Journal of Oral and Maxillofacial Surgery 55, 1287, 1997.
24. Froum S.J., Wallace S.S., Elian N., Cho S.C., and Tarnow D.P. Comparison of mineralized cancellous bone allograft (Puros) and anorganic bovine bone matrix (Bio-Oss) for sinus augmentation: histomorphometry at 26 to 32 weeks after grafting. International Journal of Periodontics & Restorative Dentistry 26, 543, 2006.
25. Kolerman R., Tal H., and Moses O. Histomorphometric analysis of newly formed bone after maxillary sinus floor augmentation using ground cortical bone allograft and internal collagen membrane. Journal of Periodontology 79, 2104, 2008.
26. Artzi Z., Nemcovsky C.E., and Dayan D. Bovine-HA spongiosa blocks and immediate implant placement in sinus augmentation procedures. Histopathological and histomorphometric observations on different histological stainings in 10 consecutive patients. Clinical Oral Implants Research 13, 420, 2002.
27. Merkx M.A., Maltha J.C., and Stoelinga P.J. Assessment of the value of anorganic bone additives in sinus floor augmentation: a review of clinical reports. International Journal of Oral and Maxillofacial Surgery 32, 1, 2003.
28. Schwartz Z., Weesner T., van Dijk D.S., Cochran D.L., Mellonig J.T., Lohmann C.H., Carnes D.L., Goldstein M., Dean D.D., and Boyan B.D. Ability of deproteinized cancellous bovine bone to induce new bone formation. Journal of Periodontology 71, 1258, 2000.
29. Tadjoedin E.S., de Lange G.L., Holzmann P.J., Kulper L., and Burger E.H. Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. Clinical Oral Implants Research 11, 334, 2000.
30. Fetner A.E., Hartigan M.S., and Low S.B. Periodontal repair using PerioGlas in nonhuman primates: clinical and histologic observations. Compendium 15, 932, 1994.
31. Oonishi H., Kushitani S., Yasukawa E., Iwaki H., Hench L.L., Wilson J., Tsuji E., and Sugihara T. Particulate bioglass compared with hydroxyapatite as a bone graft substitute. Clinical Orthopaedics and Related Research 334, 316, 1997.
32. Desjardins R.P. Hydroxyapatite for alveolar ridge augmentation: indications and problems. Journal of Prosthetic Dentistry 54, 374, 1985.
33. Boyne P.J. Advances in preprosthetic surgery and implantation. Current Opinion in Dentistry 1, 277, 1991.
34. Begley C.T., Doherty M.J., Mollan R.A., and Wilson D.J. Comparative study of the osteoinductive properties of bioceramic, coral and processed bone graft substitutes. Biomaterials 16, 1181, 1995.
35. Damien C.J., Ricci J.L., Christel P., Alexander H., and Patat J.L. Formation of a calcium phosphate-rich layer on absorbable calcium carbonate bone graft substitutes. Calcified Tissue International 55, 151, 1994.
36. Szabo G., Suba Z., Hrabak K., Barabas J., and Nemeth Z. Autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevations (2- and 3-dimensional computed tomographic, histologic, and histomorphometric evaluations): preliminary results. International Journal of Oral & Maxillofacial Implants 16, 681, 2001.
37. Zijderveld S.A., Zerbo I.R., van den Bergh J.P., Schulten E.A., and ten Bruggenkate C.M. Maxillary sinus floor augmentation using a beta-tricalcium phosphate (Cerasorb) alone compared to autogenous bone grafts. International Journal of Oral & Maxillofacial Implants 20, 432, 2005.
38. Wallace S.S. and Froum S.J. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Annual Periodontology 8, 328, 2003.
39. Zerbo I.R., de Lange G.L., Joldersma M., Bronckers A.L., and Burger E.H. Fate of monocortical bone blocks grafted in the human maxilla: a histological and histomorphometric study. Clinical Oral Implants Research 14, 759, 2003.

40. Peleg M., Garg A.K., Misch C.M., and Mazor Z. Maxillary sinus and ridge augmentations using a surface-derived autogenous bone graft. Journal of Oral and Maxillofacial Surgery 62, 1535, 2004.
41. Crespi R., Vinci R., Cappare P., Gherlone E., and Romanos G.E. Calvarial versus iliac crest for autologous bone graft material for a sinus lift procedure: a histomorphometric study. International Journal of Oral & Maxillofacial Implants 22, 527, 2007.
42. Barone A., Crespi R., Aldini N.N., Fini M., Giardino R., and Covani U. Maxillary sinus augmentation: histologic and histomorphometric analysis. International Journal of Oral & Maxillofacial Implants 20, 519, 2005.
43. Meredith N., Friberg B., Sennerby L., and Aparicio C. Relationship between contact time measurements and PTV values when using the Periotest to measure implant stability. International Journal of Prosthodontics 11, 269, 1998.
44. Blomqvist J.E., Alberius P., Isaksson S., Linde A., and Hansson B.G. Factors in implant integration failure after bone grafting: an osteometric and endocrinologic matched analysis. International Journal of Oral and Maxillofacial Surgery 25, 63, 1996.
45. Jemt T. and Lekholm U. Implant treatment in edentulous maxillae: a 5-year follow-up report on patients with different degrees of jaw resorption. International Journal of Oral & Maxillofacial Implants 10, 303, 1995.
46. Groeneveld E.H., van den Bergh J.P., Holzmann P., ten Bruggenkate C.M., Tuinzing D.B., and Burger E.H. Histomorphometrical analysis of bone formed in human maxillary sinus floor elevations grafted with OP-1 device, demineralized bone matrix or autogenous bone. Comparison with non-grafted sites in a series of case reports. Clinical Oral Implants Research 10, 499, 1999.
47. Consolo U., Zaffe D., Bertoldi C., and Ceccherelli G. Platelet-rich plasma activity on maxillary sinus floor augmentation by autologous bone. Clinical Oral Implants Research 18, 252, 2007.
48. John H.D. and Wenz B. Histomorphometric analysis of natural bone mineral for maxillary sinus augmentation. International Journal of Oral & Maxillofacial Implants 19, 199, 2004.
49. Lorenzetti M., Mozzati M., Campanino P.P., and Valente G. Bone augmentation of the inferior floor of the maxillary sinus with autogenous bone or composite bone grafts: a histologic-histomorphometric preliminary report. International Journal of Oral & Maxillofacial Implants 13, 69, 1998.
50. Lundgren S., Moy P., Johansson C., and Nilsson H. Augmentation of the maxillary sinus floor with particulated mandible: a histologic and histomorphometric study. International Journal of Oral & Maxillofacial Implants 11, 760, 1996.
51. Pejrone G., Lorenzetti M., Mozzati M., Valente G., and Schierano G.M. Sinus floor augmentation with autogenous iliac bone block grafts: a histological and histomorphometrical report on the two-step surgical technique. International Journal of Oral and Maxillofacial Surgery 31, 383, 2002.
52. Raghoebar G.M., Schortinghuis J., Liem R.S., Ruben J.L., van der Wal J.E., and Vissink A. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clinical Oral Implants Research 16, 349, 2005.
53. Scarano A., Degidi M., Iezzi G., Pecora G., Piatelli M., Orsini G., Caputi S., Perrotti V., Mangano C., and Piatteli A. Maxillary sinus augmentation with different biomaterials: a comparative histologic and histomorphometric study in man. Implant Dentistry 15, 197, 2006.
54. Szabo G., Huys L., Coulthard P., Maiorana C., Garagiola U., Barabás J., Németh Z., Hrabák K., and Suba Z. A prospective multicenter randomized clinical trial of autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevation: histologic and histomorphometric evaluation. International Journal of Oral & Maxillofacial Implants 20, 371, 2005.
55. Thor A., Franke-Stenport V., Johansson C.B., and Rasmusson L. Early bone formation in human bone grafts treated with platelet-rich plasma: preliminary histomorphometric results. International Journal of Oral and Maxillofacial Surgery 36, 1164, 2007.
56. Turunen T., Peltola J., Yli-Urpo A., and Happonen R.P. Bioactive glass granules as a bone adjunctive material in maxillary sinus floor augmentation. Clinical Oral Implants Research 15, 135, 2004.
57. van den Bergh J.P., ten Bruggenkate C.M., Groeneveld H.H., Burger E.H., and Tuinzing D.B. Recombinant human bone morphogenetic protein-7 in maxillary sinus floor elevation surgery in 3

patients compared to autogenous bone grafts. A clinical pilot study. Journal of Clinical Periodontology 27, 627, 2000.

58. Zerbo I.R., Zijderveld S.A., de Boer A. Bronckers A.L., de Lange G., ten Bruggenkate C.M., and Burger E.H. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clinical Oral Implants Research 15, 724, 2004.
59. Suba Z., Takacs D., Matusovits D., Barabas J., Fazekas A., and Szabo G. Maxillary sinus floor grafting with beta-tricalcium phosphate in humans: density and microarchitecture of the newly formed bone. Clinical Oral Implants Research 17, 102, 2006.
60. Simunek A., Kopecka D., Somanathan R.V., Pilathadka S., and Brazda T. Deproteinized bovine bone versus beta-tricalcium phosphate in sinus augmentation surgery: a comparative histologic and histomorphometric study. International Journal of Oral & Maxillofacial Implants 23, 935, 2008.
61. Gerressen M., Hermanns-Sachweh B., Riediger D., Hilgers R.D., Spiekermann H., and Ghassemi A. Purely cancellous vs. corticocancellous bone in sinus floor augmentation with autogenous iliac crest: a prospective clinical trial. Clinical Oral Implants Research 20, 109, 2009.
62. Hallman M., Sennerby L., and Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. International Journal of Oral & Maxillofacial Implants 17, 635, 2002.
63. Le Lorc'h-Bukiet I., Tulasne J.F., Llorens A., and Lesclous P. Parietal bone as graft material for maxillary sinus floor elevation: structure and remodeling of the donor and of recipient sites. Clinical Oral Implants Research 16, 244, 2005.
64. Crespi R., Mariani E., Benasciutti E., Cappare P., Cenci S., and Gherlone E. Magnesium-enriched hydroxyapatite versus autologous bone in maxillary sinus grafting: combining histomorphometry with osteoblast gene expression profiles ex vivo. Journal of Periodontology 80,586, 2009.
65. Hanisch O., Lozada J.L., Holmes R.E., Calhoun C.J., Kan J.Y., and Spiekermann H. Maxillary sinus augmentation prior to placement of endosseous implants: A histomorphometric analysis. International Journal of Oral & Maxillofacial Implants 14, 329, 1999.
66. Landi L., Pretel R.W. Jr., Hakimi N.M., and Setayesh R. Maxillary sinus floor elevation using a combination of DFDBA and bovine-derived porous hydroxyapatite: a preliminary histologic and histomorphometric report. International Journal of Periodontics & Restorative Dentistry 20, 574, 2000.
67. Moy P.K., Lundgren S., and Holmes R.E. Maxillary sinus augmentation: histomorphometric analysis of graft materials for maxillary sinus floor augmentation. Journal of Oral and Maxillofacial Surgery 51, 857, 1993.
68. Noumbissi S.S., Lozada J.L., Boyne P.J., Rohrer M.D., Clem D., Kim J.S., and Prasad H. Clinical, histologic, and histomorphometric evaluation of mineralized solvent-dehydrated bone allograf (Puros) in human maxillary sinus grafts. Journal of Oral Implantology 31, 171, 2005.
69. Froum S.J., Tarnow D.P., Wallace S.S., Rohrer M.D., and Cho S.C. Sinus floor elevation using anorganic bovine bone matrix (OsteoGraf/N) with and without autogenous bone: a clinical, histologic, radiographic, and histomorphometric analysis--Part 2 of an ongoing prospective study. International Journal of Periodontics & Restorative Dentistry 18, 528, 1998.
70. Kassolis J.D. and Reynolds M.A. Evaluation of the adjunctive benefits of platelet-rich plasma in subantral sinus augmentation. Journal of Craniofacial Surgery 16, 280, 2005.
71. Stacchi C., Orsini G., Di Lorio D., Breschi L., and Di Lenarda R. Clinical, histologic, and histomorphometric analyses of regenerated bone in maxillary sinus augmentation using fresh frozen human bone allografts. Journal of Periodontology 79, 1789, 2008.
72. Choukroun J., Diss A., Simonpieri A., Girard M.O., Schoeffler C., Dohan S.L., Dohan A.J., Mouhyi J., and Dohan D.M. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics 101, 299, 2006.
73. Cammack G.V., Nevins M., Clem D.S. III, Hatch J.P., and Mellonig J.T. Histologic evaluation of mineralized and demineralized freeze-dried bone allograft for ridge and sinus augmentations.

International Journal of Periodontics & Restorative Dentistry 25, 231, 2005.

74. Lee Y.M., Shin S.Y., Kim J.Y., Kye S.B., Ku Y., and Rhyu I.C. Bone reaction to bovine hydroxyapatite for maxillary sinus floor augmentation: histologic results in humans. International Journal of Periodontics & Restorative Dentistry 26, 471, 2006.
75. Springer I.N., Nocini P.F., Schlegel K.A. De Santis D., Park J., Warnke P.H., Terheyden H., Zimmermann R., Chiarini L., Gardner K., Ferrari F., and Wiltfang J. Two techniques for the preparation of cell-scaffold constructs suitable for sinus augmentation: steps into clinical application. Tissue Engineering 12, 2649, 2006.
76. Traini T, Degidi M., Sammons R., Stanley P., and Piattelli A. Histologic and elemental microanalytical study of anorganic bovine bone substitution following sinus floor augmentation in humans. Journal of Periodontology 79, 1232, 2008.
77. Valentini P., Abensur D., Wenz B., Peetz M., and Schenk R. Sinus grafting with porous bone mineral (Bio-Oss) for implant placement: a 5-year study on 15 patients. International Journal of Periodontics & Restorative Dentistry 20, 245, 2000.
78. Wheeler S.L., Holmes R.E., and Calhoun C.J. Six-year clinical and histologic study of sinus-lift grafts. International Journal of Oral & Maxillofacial Implants 11, 26, 1996.
79. Yildirim M., Spiekermann H., Biesterfeld S., and Edelhoff D. Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood. A histologic and histomorphometric study in humans. Clinical Oral Implants Research 11, 217, 2000.
80. Froum S.J., Wallace S.S., Cho S.C., Elian N., and Tarnow D.P. Histomorphometric comparison of a biphasic bone ceramic to anorganic bovine bone for sinus augmentation: 6- to 8-month postsurgical assessment of vital bone formation. A pilot study. International Journal of Periodontics & Restorative Dentistry 28, 273, 2008.
81. Artzi Z., Nemcovsky C.E., Tal H., and Dayan D. Histopathological morphometric evaluation of 2 different hydroxyapatite-bone derivatives in sinus augmentation procedures: a comparative study in humans. Journal of Periodontology 72, 911, 2001.
82. Mangano C., Scarano A., Perrotti V., Iezzi G., and Piattelli A. Maxillary sinus augmentation with a porous synthetic hydroxyapatite and bovine-derived hydroxyapatite: a comparative clinical and histologic study. International Journal of Oral & Maxillofacial Implants 22, 980, 2007.
83. Orsini G., Scarano A., Piattelli M., Piccirilli M., Caputi S., and Piattelli A. Histologic and ultrastructural analysis of regenerated bone in maxillary sinus augmentation using a porcine bone-derived biomaterial. Journal of Periodontology 77, 1984, 2006.
84. Cordaro L., Bosshardt D.D., Palattella P., Rao W., Serino G., and Chiapasco M. Maxillary sinus grafting with Bio-Oss or Straumann Bone Ceramic: histomorphometric results from a randomized controlled multicenter clinical trial. Clinical Oral Implants Research 19, 796, 2008.
85. Ozyuvaci H., Bilgic B., and Firatli E. Radiologic and histomorphometric evaluation of maxillary sinus grafting with alloplastic graft materials. Journal of Periodontology 74, 909, 2003.
86. Cordioli G., Mazzocco C., Schepers E., Brugnolo E., and Majzoub Z. Maxillary sinus floor augmentation using bioactive glass granules and autogenous bone with simultaneous implant placement. Clinical and histological findings. Clinical Oral Implants Research 12, 270, 2001.
87. Galindo-Moreno P., Avila G., Fernandez-Barbero J.E., Mesa F., O'Valle-Ravassa F., and Wang H.L. Clinical and histologic comparison of two different composite grafts for sinus augmentation: a pilot clinical trial. Clinical Oral Implants Research 19, 755, 2008.
88. Mangano C., Scarano A., Iezzi G., Orsini G., Perrotti V., Mangano F., Montini S., Piccirilli M., and Piattelli A. Maxillary sinus augmentation using an engineered porous hydroxyapatite: a clinical, histological, and transmission electron microscopy study in man. Journal of Oral Implantology 32, 122, 2006.
89. Artzi Z., Nemcovsky C.E., and Dayan D. Nonceramic hydroxyapatite bone derivative in sinus augmentation procedures: clinical and histomorphometric observations in 10 consecutive cases. International Journal of Periodontics & Restorative Dentistry 23, 381, 2003.
90. Canullo L., and Dellavia C. Sinus Lift Using a Nanocrystalline Hydroxyapatite Silica Gel in Severely Resorbed Maxillae: Histological Preliminary Study. Clinical Implant Dentistry and Related Research 11 Suppl 1, 7, 2009.

91. Ewers R. Maxilla sinus grafting with marine algae derived bone forming material: a clinical report of long-term results. Journal of Oral and Maxillofacial Surgery 63, 1712, 2005.
92. Simunek A., Cierny M., Kopecka D., Kohout A., Bukac J., and Vahalova D. The sinus lift with phycogenic bone substitute. A histomorphometric study. Clinical Oral Implants Research 16, 342, 2005.
93. Knabe C., Koch C., Rack A., and Stiller M. Effect of beta-tricalcium phosphate particles with varying porosity on osteogenesis after sinus floor augmentation in humans. Biomaterials 29, 2249, 2008.
94. Zerbo I.R., Bronckers A.L., de Lange G.L., van Beek G.J., and Burger E.H. Histology of human alveolar bone regeneration with a porous tricalcium phosphate. A report of two cases. Clinical Oral Implants Research 12, 379, 2001.
95. Artzi Z., Kozlovsky A., Nemcovsky C.E., and Weinreb M. The amount of newly formed bone in sinus grafting procedures depends on tissue depth as well as the type and residual amount of the grafted material. Journal of Clinical Periodontology 32, 193, 2005.
96. Hallman M., Lundgren S., and Sennerby L. Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. Clinical Implant Dentistry and Related Research 3, 87, 2001.
97. Tadjoedin E.S., de Lange G.L., Bronckers A.L., Lyaruu D.M., and Burger E.H. Deproteinized cancellous bovine bone (Bio-Oss) as bone substitute for sinus floor elevation. A retrospective, histomorphometrical study of five cases. Journal of Clinical Periodontology 30, 261, 2003.
98. Yildirim M., Spiekermann H., Handt S., and Edelhoff D. Maxillary sinus augmentation with the xenograft Bio-Oss and autogenous intraoral bone for qualitative improvement of the implant site: a histologic and histomorphometric clinical study in humans. International Journal of Oral & Maxillofacial Implants 16, 23, 2001.
99. Marx R.E., Carlson E.R., Eichstaedt R.M., Schimmele S.R., Strauss J.E., and Georgeff K.R. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics 85, 638, 1998.
100. Boyapati L. and Wang H.L. The role of platelet-rich plasma in sinus augmentation: a critical review. Implant Dentistry 15, 160, 2006.
101. Plachokova A.S., Nikolidakis D., Mulder J., Jansen J.A, and Creugers N.H. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. Clinical Oral Implants Research 19, 539, 2008.
102. Horch H.H. and Steegmann B. Experience with resorbable TCP-ceramic granules for the filling of large bone defects after cystectomy in the jaw. deutsche zahnärztliche zeitschrift 40, 672, 1985.
103. Hallman M., Cederlund A., Lindskog S., Lundgren S., and Sennerby L. A clinical histologic study of bovine hydroxyapatite in combination with autogenous bone and fibrin glue for maxillary sinus floor augmentation. Results after 6 to 8 months of healing. Clinical Oral Implants Research 12, 135, 2001.
104. Schepers E., de Clercq C.M., Ducheyne P., and Kempeneers R. Bioactive glass particulate material as a filler for bone lesions. Journal of Oral Rehabilitation 18, 439, 1991.
105. Jensen O.T. and Sennerby L. Histologic analysis of clinically retrieved titanium microimplants placed in conjunction with maxillary sinus floor augmentation. International Journal of Oral & Maxillofacial Implants 13, 513, 1998.
106. Raghoebar G.M., Batenburg R.H., Timmenga N.M., Vissink A., and Reintsema H. Morbidity and complications of bone grafting of the floor of the maxillary sinus for the placement of endosseous implants. Mund Kiefer Gesichtschirurgie 3 Suppl 1, 65, 1999.
107. Verhoeven J.W., Cune M.S., Terlou M, Zoon M.A., and de Putter C. The combined use of endosteal implants and iliac crest onlay grafts in the severely atrophic mandible: a longitudinal study. International Journal of Oral and Maxillofacial Surgery 26, 351, 1997.
108. Triplett R.G., Schow S.R., and Laskin D.M. Oral and maxillofacial surgery advances in implant dentistry. International Journal of Oral & Maxillofacial Implants 15, 47, 2000.
109. Schlegel K.A., Schultze-Mosgau S., Wiltfang J., Neukam F.W., Rupprecht S., and Thorwarth M.

Changes of mineralization of free autogenous bone grafts used for sinus floor elevation. Clinical Oral Implants Research 17, 673, 2006.

110. Becktor J.P., Hallstrom H., Isaksson S., and Sennerby L. The use of particulate bone grafts from the mandible for maxillary sinus floor augmentation before placement of surface-modified implants: results from bone grafting to delivery of the final fixed prosthesis. Journal of Oral and Maxillofacial Surgery 66, 780, 2008.

111. Yamamichi N., Itose T., Neiva R., and Wang H.L. Long-term evaluation of implant survival in augmented sinuses: a case series. International Journal of Periodontics & Restorative Dentistry 28, 163, 2008.

112. Tarnow D.P., Wallace S.S., Froum S.J., Rohrer M.D., and Cho S.C. Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: Part 3 of an ongoing prospective study. International Journal of Periodontics & Restorative Dentistry 20, 117, 2000.

113. Avera S.P., Stampley W.A., and McAllister B.S. Histologic and clinical observations of resorbable and nonresorbable barrier membranes used in maxillary sinus graft containment. International Journal of Oral & Maxillofacial Implants 12, 88, 1997.

# CHAPTER 04

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# CHAPTER 04

## Predictive value of ridge dimensions on autologous bone graft resorption in staged maxillary sinus augmentation surgery using Cone Beam CT

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# INTRODUCTION

After loss of a single tooth or multiple teeth, the morphology of the alveolar crest changes. Bone reduction in both corono-apical and bucco-lingual direction has been observed,[1;2] which significantly limits the insertion of implants of desired length and diameter.[3;4] Reduced residual bone dimensions combined with pneumatization of the maxillary sinus is considered to be an indication for augmentation procedures.[5;6] Previous studies estimated these dimensional changes of the alveolar crest after tooth loss as well as their relationship to the maxillary sinus, but these evaluations were based on panoramic radiographs.[5;7-9] Panoramic radiographs only allow a two-dimensional evaluation and distances may be affected by magnification or distortion.[10] Recently, Pramstraller et al. published the first study evaluating three-dimensional morphology of the alveolar ridge based on Computerized Tomography (CT) scans.[6] To date, conventional CT protocols are generally associated with relatively high radiation dose levels compared to two-dimensional imaging.[11] The last decade, Cone Beam Computerized Tomography (CBCT) gained more popularity as CBCT provides a lower cost alternative and easy accessibility compared to conventional CT, and can therefore be used in a wider range of oral and maxillofacial patients.[12;13] Although it was also described that CBCT provides a lower radiation dose, a recent systematic review by de Vos et al. showed that there is a lack of evidence-based data to validate this statement.[12]

Several augmentation techniques have been developed to increase the amount of bone to allow functional dental implant placement in an atrophic posterior maxilla.[14] Currently, sinus floor augmentation is the most-common and widely accepted augmentation method.[15;16] With respect to the augmentation material, although a broad range of bone substitutes is used in sinus floor augmentation procedures, autologous bone grafts are still considered the gold standard.[17;18] Nevertheless, resorption and remodeling of an autologous bone graft is still a concern, as an imbalance between these processes may lead to insufficient bone volume thereby frustrating implant installation.[19] Previous reports have clearly indicated that volume reduction of the transplanted bone results in an increased implant loss in one-stage procedures or could affect the positioning of implants in two-stage procedures in cases, in which an insufficient amount of transplanted bone remains.[19-21] Furthermore, bone volume resorption around implants is described to be a continuous problem, as the processes of resorption and remodeling of transplanted bone may persist for years.[23;24]

Besides ridge dimensions, also bone graft resorption was quantified using two dimensional panoramic images.[9;25] However, with the introduction of (CB)CT imaging, calculation of the absolute quantity of bone grafted within the maxil-

lary sinus as well as the dimensions of the alveolar ridge is nowadays possible.[21;26;27] Volumetric reduction of grafting material was observed using both autologous bone graft as well as synthetic bone substitute materials. Johansson et al. evaluated volume change of autologous bone grafts, for which an average volume loss of approximately 50% was found.[27] Wanschitz et al. evaluated algae-derived hydroxyapatite for sinus floor augmentation which resulted in an average volume loss of around 14%.[21] Kirmeier et al. used mainly Bio Oss and found a overall reduction in the order of 25%.[26]

Besides bone volume, also bone quality appears important for primary implant stability and clinical outcome.[20] Adequate primary implant stability is often difficult to achieve in low quality bone, resulting in a significant decrease of implant success rate over time.[28;29] Bone graft quality is mostly expressed in terms of bone density. However, factors such as bone metabolism, cell turn over, mineralization, maturization, intercellular matrix and vascularity are also important in the definition of bone quality and also may influence clinical outcome.[22] As an additional advantage of using CBCT imaging over panoramic radiographs, bone density can also be determined, however not in Hounsfield Unit (HU), as is the case in conventional CT.[30] These HU units are based on density values for air (–1,000 HU) and pure water (0 HU). A decrease in density results from decalcification and reduction of cortical and trabecular bone. Therefore, bone density has been correlated to the quality of the bone graft.[31]

Although resorption of bone grafts after maxillary sinus augmentation has been described radiographically, no studies are available providing predictive parameters about the expected amount of resorption of transplanted bone grafts. Therefore, the aim of this study was to determine which parameters influence the outcome of autologous bone graft resorption. The hypothesis was that especially the original dimensions of the surrounding bone (i.e. the alveolar crest and maxillary sinus) in which an autologous bone graft is placed can predict the outcome of the process of bone resorption and remodeling. Furthermore, patients' gender and age as well as graft healing time interval may also influence volume changes. The experimental set-up consisted of a retrospective three dimensional analysis of alveolar ridge dimensions and bone graft volume change in the atrophic posterior maxilla performed by CBCT imaging, follow by multi-level analysis of variables found in 20 patients.

# MATERIALS AND METHODS

### Patients and surgical procedure

A retrospective cohort design (trohoc) was used for this study. All maxillary sinus augmentation surgeries and CBCT data acquisition in the study population took place between 2006 and 2009. The study criteria were a CBCT scan directly before and within 2 weeks after maxillary sinus floor augmentation surgery, and one before implant placement. Furthermore, patients should have been at least ten years edentulous. A total of 20 consecutive edentulous Caucasian patients fulfilling these criteria (12 females with a mean age of 56 year (SD=8 year) and 8 males with a mean age of 56 year (SD=14 year) were included in this study. Of these 20 patients, 18 patients received bilateral and 2 received a unilateral sinus augmentation, resulting in a total of 38 augmented sinuses. Autologous bone blocks and granulates harvested from the iliac crest were used without any additive. In some cases, additional screw fixed onlay bone blocks were used to increase the alveolar crest width.

### CBCT imaging

For each patient, CBCT imaging was performed before maxillary sinus augmentation surgery (t=0), within 2 weeks after maxillary sinus floor augmentation surgery (t=1), and after a graft healing interval of several months (median=4 months; t=2). The pre-operative and post-operative scans were acquired using the i-CAT® 3D Imaging System (Imaging Sciences International Inc, Hatfield, PA, USA) with a field of view of 22×16 cm and 0.4 mm voxel size. For further CBCT imaging specification see Table 1.

| | |
|---|---|
| X-ray source | High frequency, constant potential, fixed anode 120 kVp, 3-8 mA (pulse mode) |
| X-ray beam | Cone-beam |
| Focal spot | 0.5 mm |
| Field of view | 16 cm (diameter) * 22 cm (height) |
| Image detector | Amorphous silicon flat panel 20 cm * 25 cm |
| Voxel size | 0.4 $mm^3$ |
| Gray scale | 16 bit |
| Scan time | 20 seconds (or 40 seconds for extended height) |
| Radiation dose | Maximal 136 µSv for extended height |

Table 1: i-CAT™ 3D imaging system specifications

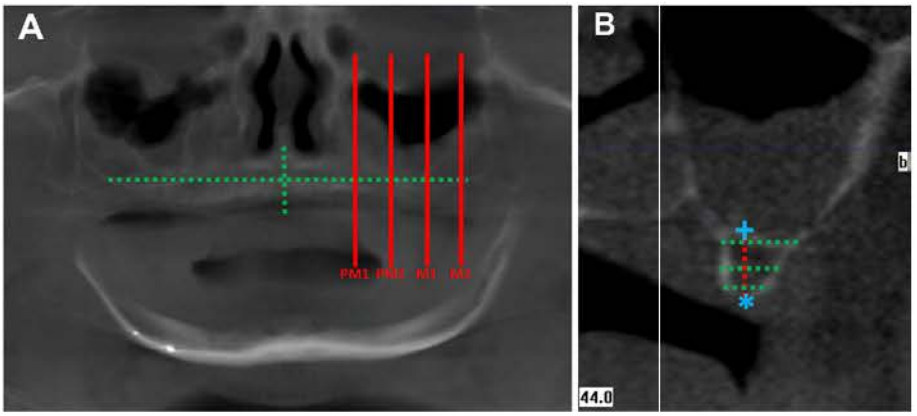


Figure 1: Alveolar ridge dimension measurement. A) On a panoramic slice of a preoperative CBCT scan (t=0) of an edentulous patients, a section of interest (SOI) was selected at both sites at 21, 28, 36 and 43 mm, and 22, 29, 37 and 44 mm from the incisive foramen, respectively in females and males. SOIs allocated the area of the first premolar (PM1), second premolar (PM2), first molar (M1) and second molar (M2). B) Example of bone height (BH) measurement between the most coronal point of the alveolar crest (*) and the most coronal point of the sinus floor (+). Bone width (BW) was evaluated at a height of 1, 3, and 7 mm apically of (*).

### Alveolar crest dimension analysis

Pre-operative CBCT scans (t=0) were assessed in I-Cat Vision® software (Imaging Sciences International, Inc. Hatfield, PA, USA). Alveolar crest dimensions before sinus floor augmentation were evaluated at four regions (i.e. first and second molars and premolars regions) per edentulous site according the method of Pramstraller et al..[6] Shortly, the mean distance from the incisive foramen to the CT cross-section of the first premolar, second premolar, first molar and second molar was 21.2, 28.2, 36.1 and 44.0 mm, respectively in females and 22.0, 29.0, 37.1 an 45.0, respectively, in males (Figure 1a). The CT cross-section for each premolar and molar region was regarded as the section of interest (SOI). At the SOI, the alveolar bone height (BH) was measured between the most coronal point of the alveolar crest ($h_{crest}$) and the most coronal point of the sinus floor ($h_{sinus}$) (Figure 1b). At a height of exact 1.0, 3.0, and 7.0 mm apically of $h_{crest}$, bone width (BW) was measured as the width of the alveolar crest (Figure 1b).

### Bone graft volume analysis

Post-operative CBCT scans (t=1 and t=2) were processed according to the method described by Cuijpers et al. to evaluate calcified bone graft volume changes.[32] Shortly, I-CAT images were converted into a stack of Digital Imaging and Communications in Medicine (DICOM) format image files and converted from 16 -bit gray scale to 8-bit gray scale images using 3D-Doctor software V4 (Able Software Corp, Lexiton, MA, USA). The files were imported in CT-Analyzer V1.10 (Skyscan; Kontich, Belgium) and re-sliced into coronal sections. Section thickness

was 0.265 mm between resliced images. The three dimensional volume of interest (VOI), consisting of the bone graft located inside the maxillary sinus, was manually drawn and interpolated through consecutive coronal sections (Figure 2a). Grafted bone could be distinguished from original bone by density and shape of the maxillary sinus. Care was taken not to include local original bone. After selection of the VOI, calcified grafted bone was differentiated from closely related soft tissues and fluids by gray value selection (Figure 2b). By setting the threshold relatively high (i.e. at least a gray value of 110), only dense calcified bone suggestive for a high quality was selected. Moreover, comparison between local original bone and grafted bone was performed to set the band width. CT-Analyzer was used to calculate the absolute amount of selected bone within the VOI. Calcified bone graft volume differences between t=1 and t=2 were denoted as 'bone volume change' and expressed in terms of percentage.

**Statistical data analysis**

Mean and standard deviation (SD) were calculated for comparable measurements to reduce the number of possible variables for further analysis. Pearson correlation testing (IBM® SPSS 16.0) was performed to assess if it was statistically acceptable to cluster these measurements. By this method, the number of variables was reduced with only marginal loss of information by using the mean premolar BW ($BW_{pm}$), mean molar BW ($BW_{m}$) and mean BH.

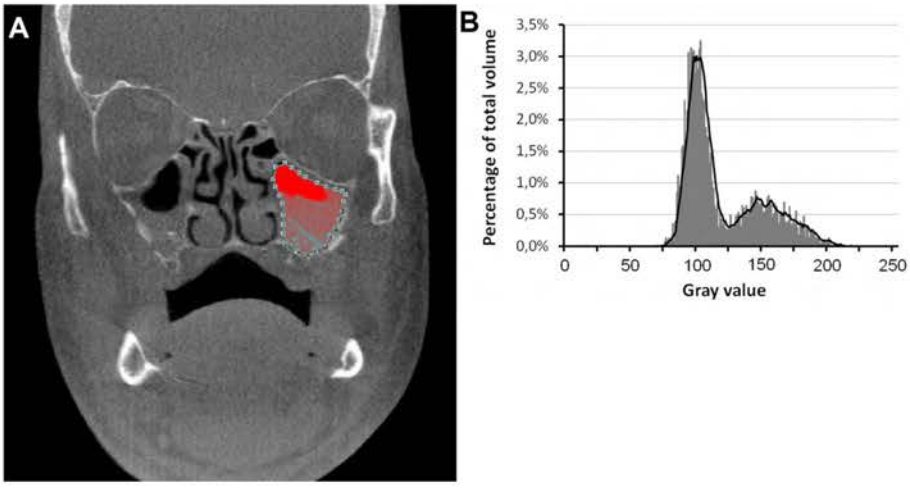


Figure 2: Graft selection and gray value. A) The volume of interest (VOI), consisting of the bone graft located inside the maxillary sinus, was manually drawn and interpolated through consecutive coronal sections. The selected area is shown in red. B) Example of gray value distribution within the VOI. A lower threshold of at least 110 gray value was used including only dense calcified bone.

To analyze the relation between 'bone volume change' with the independent variables 'gender', 'age', 'healing time interval' 'mean BH', 'mean $BW_{pm}$' and mean $BW_m$', a linear regression model was build. Since 38 observations in 20 patients were available, the data were clustered. Therefore, the multi-level extension of linear regression was applied. To further decrease the amount of variables, first each variable was univariately assessed by multi-level analysis. Only variables with a *p*-value below 0,5 were considered for further analysis. Using these selected variables, a model was created and backward elimination of variables was performed with a threshold for the *p*-value of above 0.1 for removing a variable from the model. The package R, version 2.10.1 was used for statistical analysis.

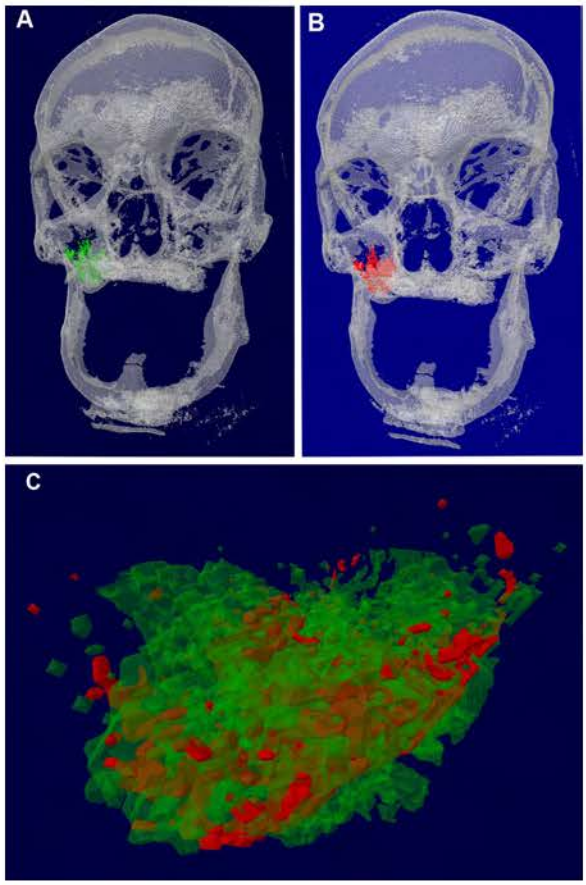


Figure 3: 3D illustration of bone graft change. A) CBCT three dimensional illustration of selected grafted bone inside the right maxillary sinus (green) within two weeks of maxillary sinus augmentation surgery (t=1). B) CBCT three dimensional illustration of selected grafted bone inside the right maxillary sinus (red) within a mean healing time of around five months after maxillary sinus augmentation surgery (t=2). C) Three dimensional overlay of both selected bone grafts (lateral view). The green (t=1) is shown with a lower transparency than red (t=2), indicating volume reduction over time.

# RESULTS

### Alveolar crest dimensions analysis

No patients were excluded. Alveolar crest dimensions were measured in pre operative CBCT scans at 38 sites in 20 consecutive edentulous patients (Figure 1a-b). Pearson correlation tests showed significance between all possible pairs of bone height measurements (data not shown). A mean residual bone height (BH) of 6.0 mm (SD=3.7 mm) was found at the left sides and 6.2 mm (SD=3.6 mm) at the right sides (Table 2). Additionally, Pearson correlation tests showed, in over 90% of all possible pairs of bone width at molar and premolar SOIs, a significant correlation (data not shown). A mean alveolar $BW_{pm}$ of 6.5 mm (SD=2.2 mm) was found at the left and 7.0 mm (SD=2.3 mm) at the right site, respectively (Table 2). At the molar region, a significant higher mean alveolar $BW_m$ of 8.8 mm (SD=2.2 mm) ($p$=0.003) and 8.9 mm (SD=2.5 mm) ($p$=0.017) at the left and right side was found compared to alveolar $BW_{pm}$, respectively (Table 2).

### Bone graft volume analysis

Bone grafts could be identified by selection on CBCT images within 2 weeks after maxillary sinus floor augmentation surgery (t=1), and after a graft healing interval ranging from 2 to 13 months (t=2) for each patient (Figure 3a-b). Bone graft volume change could be observed by transparency overlay of both VOIs (Figure 3c).

In Figure 4, mean absolute volumetric measurements of bone graft volume are depicted of CBCT scans taken at t=1 and t=2 for both left and right side grafted sinuses. Bone graft volume decrease from 735 $mm^3$ (SD=406 $mm^3$) to 540 $mm^3$ (SD=375 $mm^3$) at the left site and from 1019 $mm^3$ (SD=788 $mm^3$) to 798 $mm^3$ (SD=678 $mm^3$) at the right site. These results demonstrate a overall volume reduction of 25.0% (SD=21.0%, range: 3-78%) after 4.7 months (SD=2.7, median=4.0 months) of healing time (Figure 5).

| | *Left side* | *Right side* |
|---|---|---|
| Bone height | 6.0 ± 3.7 (median: 5.0) | 6.2 ± 3.6 (median: 6.3) |
| Bone width premolar | 6.5 ± 2.2 (median: 6.0) | 7.0 ± 2.3 (median: 7.2) |
| Bone width molar | 8.8 ± 2.2 (median: 8.3) | 8.9 ± 2.5 (median: 9.0) |

Table 2: Alveolar ridge dimensions (mean ± SD) before maxillary sinus augmentation surgery (t=0) measured by CBCT (mm).

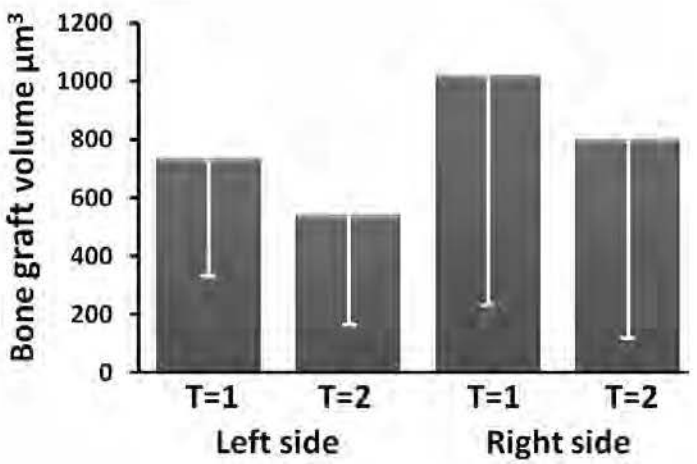


Figure 4: Bone graft volume change per site. Absolute bone graft volume change at the left and right maxillary sinus between sinus augmentation (t=1) and after 4.7 ± 2.7 months of healing time (t=2) (range: 2-13 months). Sample size: left side 18 sinuses, right side 20 sinuses.

| | Value (%) | Std.Error (%) | *p*-value |
|---|---|---|---|
| Constant | -49.3 | 25.9 | 0.07 |
| Age | 0.9 | 0.3 | 0.02 |
| Mean bone height | -2.1 | 0.8 | 0.03 |
| Mean bone width premolar | 1,4 | 1.4 | 0.31 |
| Mean bone width molar | -2,5 | 1.4 | 0.09 |

Table 3: Multilevel analysis model. First multilevel analysis model. Dependent variable was percentile bone volume change between t=1 and t=2.

### Statistical data analysis

Independent variables 'gender', 'age', 'healing time interval' 'mean BH', 'mean $BW_{pm}$' and mean $BW_{m}$' were analyzed by multi-level analysis to decrease the number of variables to a acceptable level for the amount of patients. The variables 'gender' and 'healing time interval' resulted in a *p*-value of 0.83 and 0.87 respectively, indicating no significant contribution to the model. The resulting variables were then further assessed by backward regression (Table 3). The variables 'age' and 'mean BH' remained in the model with a *p*--value of 0.01 and 0.04, respectively, indicating a significant influence on bone graft resorption (Table 4). A constant of 69.0% (SE=20.9%) was found for this backwards model. A 1.0% (SE=0.3%) decrease of bone graft resorption was found for each year the patient gets older. An increase in bone graft resorption of 1.8% (SE=0.8%) was found for each mm of original bone height before sinus floor augmentation.

# DISCUSSION

This study aimed to determine parameters that have a predictive value on autologous bone graft volume resorption after maxillary sinus floor augmentation. In a retrospective cohort of 20 patients, autologous bone graft volumes decreased with an average of 25% after 5 months of healing time. Assessment of potential predictive parameters resulted in a significant positive influence of initial alveolar bone height and a significant negative influence of patients age on bone graft resorption. This means that, if autologous bone grafts are placed in a maxillary sinus with relatively high alveolar bone height, graft resorption is increased. On the other hand, the resorption of autologous bone grafts decreases with patient age.

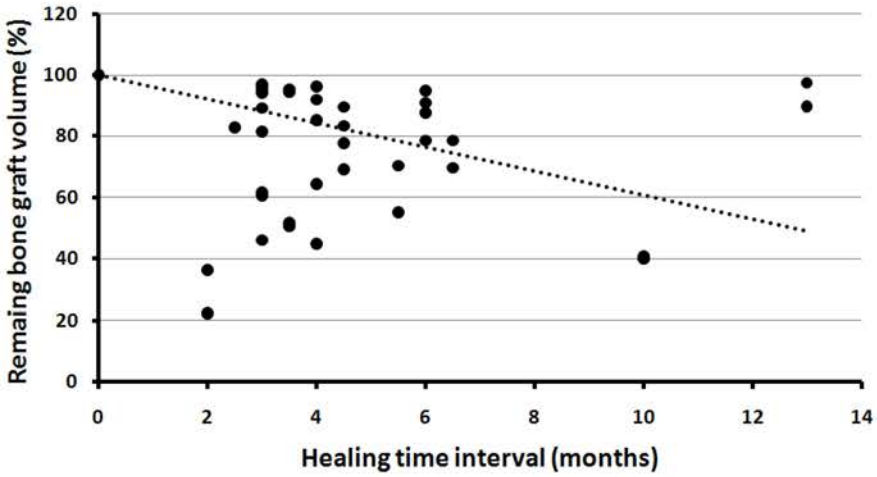


Figure 5 : Bone graft volume change in time. Scatter plot of bone graft volume change over time (range 2 to 13 months). Line indicated a non significant linear trend line. Dots indicated bone graft volume change (%) of a single maxillary sinus graft related to the initial bone graft volume (100%).

| | Value (%) | Std.Error (%) | *p*-value |
|---|---|---|---|
| Constant | -69.0 | 20.9 | 0.004 |
| Age | 1.0 | 0.3 | 0.01 |
| Mean bone height | -1.8 | 0.8 | 0.04 |

Table 4: Multilevel analysis after backward regression analysis. Dependent variable was percentile bone volume change between t=1 and t=2. A threshold for the *p*-value of above 0.1 for removing a variable from the model.

### Alveolar crest dimensions

CBCT has been shown to represent an imaging technique that can provide adequate information about alveolar ridge dimensions.[6] To obtain a basis for statistical analysis, ridge dimensions were determined by CBCT imaging only prior to sinus augmentation surgery. It has to be emphasized, that the study set-up excludes potential factors that might have contributed to earlier dimensional changes of alveolar ridge. A major factor in this respect is the time between tooth extraction and the first CBCT. Although dimensional changes in the alveolar ridge occur predominantly within the first 3 months after tooth extraction,[9] the changing of the alveolar ridge dimensions might persist for several years. In the present study, however, patients were at least ten years edentulous, thus, no further extensive resorption pattern was expected.

Pramstraller et al. concluded in a recent study, using three dimensional CT analysis, that the dimensions of the alveolar crest in the premolar and molar regions might require bone augmentation procedures in a substantial number of edentulous patients.[6] Farina et al. used the same method to compare alveolar ridge morphology between edentulous and dentulous sites, and concluded that a reduced alveolar height and bucco-lingual width was present at the edentulous sites.[2] Sinus pneumatization was found to be the cause of reduced vertical dimensions in 46% of the cases.[2]In both CT studies, alveolar bone height showed a significant decrease from the first premolar to the molar sites. In the present study, however, bone height at all four SOIs (i.e. two premolar and two molar sites) showed high correlation and consequently expressed as one variable for multilevel analysis. In contrast, bone width, showed a lower correlation between premolar and molar site and hence was included as two different variables

### Bone graft volume

In staged maxillary sinus augmentation surgery, an autologous bone graft is allowed to heal before dental implant placement. Remodeling and resorption rates of autologous bone grafts are significantly higher compared to the local original bone.[33] Long term implant survival after maxillary sinus augmentation is related to sufficient bone volume, which facilitates a reduction of intrabony stresses and stress on dental implants.[19] Monitoring the effects of sinus augmentation by volumetric measurements using CBCT has been described as an accurate method.[34] Volumetric data observed by CBCT showed high accuracy of measurement with a relative error below 1% with respect to the same measurements performed during surgery.[34] However, the selection of the ROI in separate CBCT sections remains sensitive to bias. The software used in the present study interpolated the marked areas between different consecutive slides, thereby increasing the reliability of the measurements. Furthermore, detection of bony structures might have been compromised due to the limited spatial resolution of CBCT (voxel size: 0.4 mm).

Resorption of bone grafts causes not only bone volume reduction but also decalcification and reduction of trabecular bone. Both phenomena can be analyzed and quantified using CBCT.[31;36] In this study, bone graft volume was determined by gray value selection within the VOI. By setting the threshold relatively high (i.e. at least a gray value of 110), only dense calcified bone suggestive for a high quality was selected. Furthermore, bone selected within the grafted area was compared with original bone present in the excluded surrounding area to achieve a consistent selection of calcified bone. Unfortunately, CBCT imaging and the used software package did not allow to express the selected bone graft in HU to compare these findings with earlier publications using CT. Compared to the use of CT, scatter levels and image artifacts produced by CBCT scanners are higher, and consequently the accuracy of CBCT intensity values can be affected.[36;37] However, in a recent study of Nackaerts et al. it was concluded that the intensity values derived by CBCT images are mostly influenced by the used device, imaging parameters and positioning of the patient.[37] These issues were not changed during the present study. Furthermore, a limitation for gray value selection in several patients was distortion by highly radiodense osteosynthesis material used for the buccal onlay bone blocks. Osteosynthesis material, when present within the maxillary sinus, was included in both scans resulting in an overestimation of the graft volume at both time points.

Although it seems that the mean volume of the bone graft in the right maxillary sinus was higher compared to the left site, this difference was not significant and could be explained by the fact that all surgeons were right-handed and likely graft the right maxillary sinus first. This observation was already earlier described by Johansson et al..[27] Overall measurements showed that bone graft volumes decreased by 25% within 2 to 13 months after sinus augmentation. In the past, several other studies evaluated bone graft resorption after maxillary sinus augmentation using autologous bone graft as well as synthetic bone substitute materials, and all showed volumetric reductions of the graft material.[21;26;27] Johansson et al. evaluated volume change of autologous bone grafts used for onlay or inlay purposes, for which an average volume loss of ~50% was found using a threshold of 150 HU to determine the bone graft volume changes.[27] Wanschitz et al. used algae-derived hydroxyapatite for sinus floor augmentation, but failed to differentiate bone from bone substitute material.[21] Kirmeier et al. assessed the dimensional stability of Bio-Oss or Bio-Oss combined with autologous bone after maxillary sinus augmentation.[26] A mean volume reduction of 26% was observed with a threshold of above 400 HU.[26] The present study corroborates with these reports, as a mean resorption rate of 25% was found.

### Statistical data analysis

Volumetric measurements has been described as a highly accurate method, but freehand drawings for analysis could be sensitive to analysis bias. Therefore in this study measurement of bone height and weight and also selection of the volume of interest was performed blinded by one observer. Furthermore, there is a possibility that small areas of preexisting bone were incorporated in the region of interest by selection of the volume of interest. However, the use of an experienced, blinded observer minimizes errors regarding this issue as well as bias. A multi-level analysis was performed to determine which parameters (i.e. patient gender and age, healing time interval and alveolar crest dimensions) influenced the outcome of autologous bone graft resorption. However, in this retrospective cohort study the number of patients was limited, which restricted the possibility to evaluate a large number of independent variables. As a rule of thumb 10 observations are required per variable in a regression model. To allow for multiple regression with at least three independent variables to explain loss of volume 30 observations were needed. Generally one patient will contribute two observation: left and right. This would call for a study size of 15 patients. Since in this study observations were clustered within patients, the study size was increased to 20 patients to compensate for the loss of power by clustering. Furthermore, by calculating a mean bone height over all four SOIs and a mean bone width for (pre)molar sites, the amount of parameters was substantially reduced. Then, by performing a first analysis per variable (i.e. 'gender', 'age', 'healing time interval', 'mean BH', 'mean $BW_{pm}$' and 'mean $BW_{m}$'), a selection was made based on expected significant contribution to the model. After backward analysis, only 'mean bone height' and 'age' remained in the model as significant variables influencing the amount of bone graft resorption, whereas no influence of 'gender', 'alveolar bone width' at premolar of molar sites or 'graft healing time' was found.

Thus, an increase in patient age is associated with a reduction in bone graft resorption. Therefore, it can be hypothesized that, when a higher alveolar ridge is pre-operatively present, more extensive bone augmentation is necessary to obtain sufficient bone volume after time. Or, that a lesser amount of donor bone can be harvested in a elderly patient compared to a young one. Furthermore, it can be suggested that sinus augmentation procedures in the already atrophic maxilla should preferably be performed after the majority of dimensional alveolar ridge alterations has occurred. It also can be stated that the remodeling turnover of bone transplants, both in elderly patients, as in patients in whom the residual ridge is limited, caused by whatever reason, is slowed down, implicating that more graft resorption is still to come, thus pleading for bone augmentation in time.

In addition to the parameters found in this study, a recent meta-analysis showed that total bone volumes after sinus augmentation were significantly influenced by donor site of the harvested graft, the mode of delivery of the bone graft (e.g. particulate or block), and patient age.[18] Bone grafts derived from intraoral donor sites resulted in a significant 10% higher total bone volume compared to iliac grafts, while particulation of a bone graft resulted in a significant lower amount of bone volume compared to block grafting. Thus, especially patient or graft characteristics, which are statistically proven to influence significantly the bone graft resorption process should be given full consideration when performing sinus augmentation surgery in an individual patient. Further, it can be recommended that the occurrence of continued bone graft resorption on continuated resorption after dental implant placement should be investigated further using three dimensional analyses by CBCT.

## CONCLUSION

The present study evaluated the influence of patient characteristics and alveolar ridge dimensions on changes in autologous bone graft volume after sinus augmentation based on CBCT imaging. A overall mean volume reduction of 25% was found after ~4 months of healing time. Alveolar ridge height and patient age were identified as parameters that significantly influence the decrease of bone graft volume. Thus, if autologous bone grafts are placed in a maxillary sinus with relatively high alveolar bone height, graft resorption is increased. On the other hand, the resorption of autologous bone grafts decreases with patient age. Consequently, patient characteristics that affect the process of bone graft resorption should be given full consideration when performing sinus augmentation surgery.

# REFERENCES


1. Schropp L., Wenzel A., Kostopoulos L., and Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. International Journal of Periodontics and Restorative Dentistry 23, 313, 2003.
2. Farina R., Pramstraller M., Franceschetti G., Pramstraller C., and Trombelli L.. Alveolar ridge dimensions in maxillary posterior sextants: a retrospective comparative study of dentate and edentulous sites using computerized tomography data. Clinical Oral Implants Research 22, 1138, 2011.
3. Eufinger H., Konig S., and Eufinger A. The role of alveolar ridge width in dental implantology. Clinical Oral Investigations 1, 169, 1997.
4. Eufinger H., Konig S., Eufinger A., and Machtens E. Significance of the height and width of the alveolar ridge in implantology in the edentulous maxilla. Analysis of 95 cadaver jaws and 24 consecutive patients. Mund Kiefer Gesichtschirurgie 3 (Suppl 1), S14, 1999.
5. Sharan A. and Madjar D. Maxillary sinus pneumatization following extractions: a radiographic study. The International Journal of Oral & Maxillofacial Implants 23, 48, 2008.
6. Pramstraller M., Farina R., Franceschetti G., Pramstraller C., and Trombelli L. Ridge dimensions of the edentulous posterior maxilla: a retrospective analysis of a cohort of 127 patients using computerized tomography data. Clinical Oral Implants Research 22, 54, 2001.
7. Saglam A.A. The vertical heights of maxillary and mandibular bones in panoramic radiographs of dentate and edentulous subjects. Quintessence International 33, 433, 2002.
8. Juodzbalys G. and Raustia A.M. Accuracy of clinical and radiological classification of the jawbone anatomy for implantation--a survey of 374 patients. Journal of Oral Implantology 30, 30, 2004.
9. Van Der Weijden F., Dell'Acqua F., and Slot D.E. Alveolar bone dimensional changes of post-extraction sockets in humans: a systematic review. Journal of Clinical Periodontology 36, 1048, 2009.
10. Bolin A., Eliasson S., von Beetzen M., and Jansson L. Radiographic evaluation of mandibular posterior implant sites: correlation between panoramic and tomographic determinations. Clinical Oral Implants Research 7, 354, 1996.
11. Loubele M., Bogaerts R., Van Dijck E., Pauwels R., Vanheusden S., Suetens P., Marchal G., Sanderink G., and Jacobs R. Comparison between effective radiation dose of CBCT and MSCT scanners for dentomaxillofacial applications. European Journal of Radiology 71, 461, 2009.
12. De Vos W., Casselman J., and Swennen G.R.J. Cone-beam computerized tomography (CBCT) imaging of the oral and maxillofacial region: A systematic review of the literature. International Journal of Oral and Maxillofacial Surgery 38, 609, 2009.
13. Ghaeminia H., Meijer G.J., Soehardi A., Borstlap W.A., Mulder J., Vlijmen O.J., Berge S.J., and Maal T.J. The use of cone beam CT for the removal of wisdom teeth changes the surgical approach compared with panoramic radiography: a pilot study. The International Journal of Oral and Maxillofacial Surgery 40, 834, 2011.
14. McAllister B.S. and Haghighat K. Bone augmentation techniques. Journal of Periodontology 78, 377, 2007.
15. Wallace S.S. and Froum S.J. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Annals of Periodontology 8, 328, 2003.
16. Nkenke E. and Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clinical Oral Implants Research 20 (Suppl 4), 124, 2009.
17. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. A Meta-Analysis of Histomorphometric Results and Graft Healing Time of Various Biomaterials Compared to Autologous Bone Used as Sinus Floor Augmentation Material in Humans. Tissue Engineering Part B Reviews 16, 493, 2010.
18. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. Sinus floor augmentation surgery using autologous bone grafts from various donor sites: a meta-analysis of the total bone volume. Tissue Engineering Part B Reviews 16, 295, 2010.

19. Tepper G., Haas R., Zechner W., Krach W., and Watzek G. Three-dimensional finite element analysis of implant stability in the atrophic posterior maxilla: a mathematical study of the sinus floor augmentation. Clinical Oral Implants Research 13, 657, 2002.
20. Esposito M., Hirsch J.M., Lekholm U., and Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (I). Success criteria and epidemiology. European Journal of Oral Sciences 106, 527, 1998.
21. Wanschitz F., Figl M., Wagner A., and Rolf E. Measurement of volume changes after sinus floor augmentation with a phycogenic hydroxyapatite. The International Journal of Oral & Maxillofacial Implants 21, 433, 2006.
22. Molly L. (2006) Bone density and primary stability in implant therapy. Clinical Oral Implants Research 17 (Suppl 2), 124, 2006.
23. Verhoeven J.W., Cune M.S., Terlou M., Zoon M.A., and de Putter C. The combined use of endosteal implants and iliac crest onlay grafts in the severely atrophic mandible: a longitudinal study. The International Journal of Oral and Maxillofacial Surgery 26, 351, 1997.
24. Zijderveld S.A., Schulten E.A., Aartman I.H., and Ten Bruggenkate C.M. Long-term changes in graft height after maxillary sinus floor elevation with different grafting materials: radiographic evaluation with a minimum follow-up of 4.5 years. Clinical Oral Implants Research 20, 691, 2009.
25. Nystrom E., Ahlqvist J., Kahnberg K.E., and Rosenquist J.B. Autogenous onlay bone grafts fixed with screw implants for the treatment of severely resorbed maxillae. Radiographic evaluation of preoperative bone dimensions, postoperative bone loss, and changes in soft-tissue profile. The International Journal of Oral and Maxillofacial Surgery 25, 351, 1996.
26. Kirmeier R., Payer M., Wehrschuetz M., Jakse N., Platzer S., and Lorenzoni M. Evaluation of three-dimensional changes after sinus floor augmentation with different grafting materials. Clinical Oral Implants Research 19, 366, 2008.
27. Johansson B., Grepe A., Wannfors K., and Hirsch J.M. A clinical study of changes in the volume of bone grafts in the atrophic maxilla. Dentomaxillofacial Radiology 30, 157, 2001.
28. Hutton J.E., Heath M.R., Chai J.Y., Harnett J., Jemt T., Johns R.B., McKenna S., McNamara D.C., Van Steenberghe D., and Taylor R. Factors related to success and failure rates at 3-year follow-up in a multicenter study of overdentures supported by Branemark implants. The International Journal of Oral & Maxillofacial Implants 10, 33, 1995.
29. Martinez H., Davarpanah M., Missika P., Celletti R., and Lazzara R. Optimal implant stabilization in low density bone. Clinical Oral Implants Research 12, 423, 2001.
30. Isoda K., Ayukawa Y., Tsukiyama Y., Sogo M., Matsushita Y., and Koyano K. Relationship between the bone density estimated by cone-beam computed tomography and the primary stability of dental implants. Clinical Oral Implants Research 2011
31. Norton M.R. and Gamble C. Bone classification: an objective scale of bone density using the computerized tomography scan. Clinical Oral Implants Research 12, 79, 2001.
32. Cuijpers V.M., Walboomers X.F., and Jansen J.A. Three-dimensional localization of implanted biomaterials in anatomical and histological specimens using combined X-ray computed tomography and three-dimensional surface reconstruction: a technical note. Tissue Engineering Part C Methods 16, 63, 2010.
33. Burchardt H. The biology of bone graft repair. Clinical Orthopaedics and Related Research 174, 28, 1983.
34. Stratemann S.A., Huang J.C., Maki K., Miller A.J., and Hatcher D.C. Comparison of cone beam computed tomography imaging with physical measures. Dentomaxillofacial Radiology 37, 80, 2008.
35. Smolka W., Eggensperger N., Carollo V., Ozdoba C., and Lizuka T. Changes in the volume and density of calvarial split bone grafts after alveolar ridge augmentation. Clinical Oral Implants Research 17, 149, 2006.
36. Meas F., Colligonon A., Vandermeulen D., Marchal G., and Suetens P. Multimodality image registration by maximization of mutual information. IEEE Transactions on Medical Imaging 16, 187, 1997.

37. Nackaerts O., Maes F., Yan H., Couto Souza P., Pauwels R., and Jacobs R. Analysis of intensity variability in multislice and cone beam computed tomography. Clinical Oral Implants Research 22, 873, 2011.

# CHAPTER 05

Tissue Engineering Part A
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# CHAPTER 05

## Three different strategies to obtain porous calcium phosphate cements: Comparison of performance in a rat skull bone augmentation model

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# INTRODUCTION

Preprosthetic surgery has become a routine procedure to obtain sufficient bone quantity and quality for dental implant installation in patients with an initial inadequate bone volume.[1] Various approaches are used to augment bone at the implantation site. The most frequently applied methods include distraction osteogenesis, guided bone regeneration and onlay bone grafting.[1] Although these procedures result in comparable levels of long-term dental implant survival,[2] a high complication rate has been reported for distraction osteogenesis and guided bone regeneration, which includes basal bone fractures, non-union of the alveolar bone, and secondary infections.[3;4] In view of these drawbacks, autologous bone onlay or inlay grafting is still the preferred bone augmentation technique.[5] Unfortunately, harvesting of an autologous bone graft is associated with serious drawbacks, such as donor site morbidity and prolonged operation time. Various synthetic bone substitutes have been developed to overcome these problems. Mostly, they are composed of calcium phosphate (CaP) ceramics.[6-10] Clinically relevant advantages of these bone substitutes compared to autologous bone are their reduced costs, safety and off the shelf availability.

Bone substitutes are mostly applied as blocks or granules.[6] Main disadvantages regarding blocks and granulated grafts include lack of shaping, difficult to handle and potential soft tissue damage.[11] As a solution for this problem, Brown and Chow introduced calcium phosphate cement (CPC) for non load bearing applications.[12] Such a CPC consists of a CaP-powder component and a liquid phase, which upon mixing allows injection of the material in combination with optimal defect filling and shaping.

Injectability, cohesion, and setting time are critical characteristics for the reliable application of CPCs.[13] In view of biological performance, important CPC properties include biocompatibility, osteoconductivity and interconnected porosity. The latter property is not only important for ingrowth of bone tissue, but is also related to the CPC degradation process.[14] Ideally for clinical applications, CPC degradation is in optimal balance with bone formation, which means that the space as becomes available during CPC degradation is directly filled up with newly-formed bone tissue. CPC degradation can take place by passive physicochemical dissolution and active cellular degradation.[15-18] Substantial efforts have been dedicated to control CPC degradation by introducing porosity within CPCs using porogens, hydrogels and hydrophobic liquids.[13] A promising method is also the introduction of interconnected porosity by inducing $CO_2$ bubbles during setting of CPC.[19;20] When entrapped during cement setting, these bubbles result in instantaneously porous CPC (CPC-IP). In vivo results of such CPC-IP, as applied in critical size bone defects, showed substantial bone formation and CPC

degradation already after 2 weeks.[20] Nevertheless, it has to be noticed that this method does not allow a good control of the pore size. A method, which can solve this issue, is the incorporation of degradable polymeric (e.g. poly(lactic-co-glycolic acid), PLGA) microspheres.[21-25] Degradation of the PLGA microspheres results in a so-called delayed porous CPC. In addition to their morphological contribution to create porosity, the released acidic by-products during degradation of the PLGA microspheres stimulate further the passive degradation of the CPC mass.[26] It can even be hypothesized that the use of dense PLGA microspheres (dPLGA) vs. hollow ones (hPLGA) will result in an additional increase of acidic by-products due to the increased amount of PLGA, which in turn will enhance the passive degradation of the CPC.

In view of the above mentioned, the aim of the present study was to compare the degradation and bone conductive capacity of CPCs with instantaneous porosity generated by $CO_2$ bubbles or delayed porosity generated by PLGA-microsphere incorporation (either hPLGA or dPLGA) as bone substitute materials for augmentation purposes using a rat skull bone augmentation model.

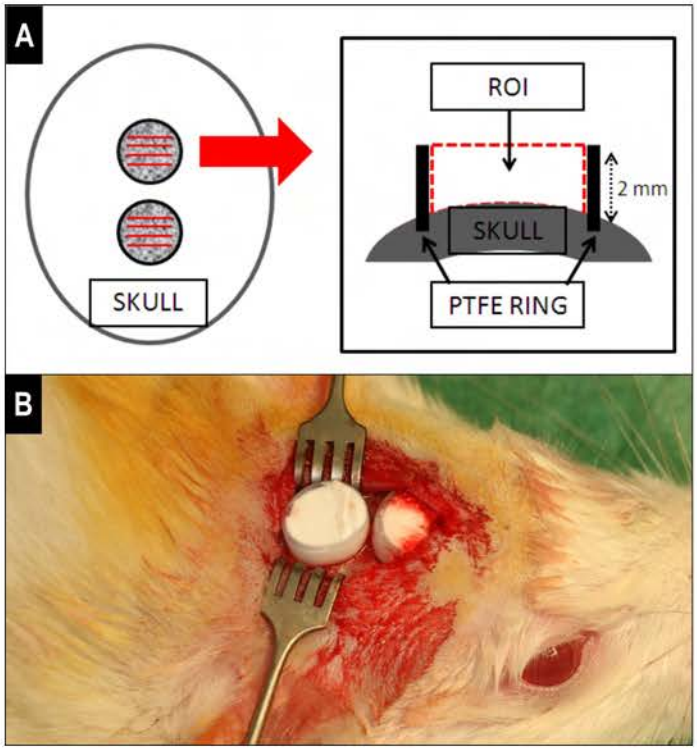


Figure 1: Surgery and histological sectioning. (a) Schematic illustration of augmentation procedure on the skull of rats using a PTFE ring filled with CPC. Red lines indicate the plane of sectioning; area bordered by dashed line indicates the region of interest (ROI). (b) Per operative illustration of two PTFE rings filled with two different CPCs augmented on top of the rat skull.

# MATERIALS AND METHODS

## Materials

Calcium phosphate cement (CPC) consisted of 85% alpha-tricalcium phosphate (Cam Bioceramics BV, Leiden, the Netherlands), 10% dicalcium phosphate dehydrate (Baker, Griesheim, Germany), and 5% precipitated hydroxyapatite (Merck, Darmstadt, Germany). For CPC-IP, 10 wt.% $NaHCO_3$ (Merck) was added. The applied liquid component was a filter-sterilized (0.2-µm) aqueous solution of $Na_2HPO_4$ (Merck).

Purasorb® PDLG 5002A (Purac, Gorinchem, The Netherlands) with an acid terminated end-group functionalization, a lactic to glycolic acid ratio of 50:50 and an average molecular weight of 17 kDa was used for the preparation of both hollow and dense poly(DL-lactic-*co*-glycolic acid) (PLGA) microspheres.

CPC-IP and CPC-PLGA composite powders were sterilized using gamma irradiation (25 kGy; Isotron B.V., Ede, The Netherlands). Polytetrafluorethylene (PTFE) rings (Polyfluor, Ede, the Netherlands) with an outer diameter (OD) of 7.0 mm, an inner diameter (ID) of 6.0 mm and a height of 2.3 mm were sterilized by autoclavation.

## Preparation of PLGA microspheres

Hollow PLGA (hPLGA) microspheres were prepared following a previously described double-emulsion solvent-extraction technique ([water in oil] in water).[21] Briefly, hPLGA microspheres were produced by injecting 500 ml of $ddH_2O$ into a tube containing a solution of 1.0 g of PLGA in 4 ml of dichloromethane. The mixture was emulsified for 90 s at 8000 rpm with an ultra turrax (IKA, Staufen, Germany). Thereafter, 6 ml of 0.3% aqueous poly (vinyl alcohol) (Acros Organics, Geel, Belgium) solution was added and emulsified for another 90 s to produce the second emulsion. This mixture was added to 394 ml of 0.3% poly (vinyl alcohol) solution and 400 ml of 2% isopropyl alcohol solution and was stirred for 2 h. The evaporation of the solvent resulted in precipitation of the dissolved polymer and hPLGA microspheres were subsequently formed. The hPLGA microspheres were allowed to settle for 1.5 h, and the solution was decanted. Then the hPLGA microspheres were collected through centrifugation at 1500 rpm for 5 min and freeze dried for 24 h.

Dense PLGA (dPLGA) microspheres were produced by Microsieve™ emulsification technology as described previously [27] and kindly provided by Nanomi (Oldenzaal, the Netherlands).

### Preparation of CPC-hPLGA and CPC-dPLGA

Density calculations of hPLGA and dPLGA microspheres indicated a 1.94-fold weight difference for dPLGA microspheres. The preparation of CPC-hPLGA was performed using 20 wt.% hPLGA; for CPC-dPLGA, similar microsphere volume was obtained using 38.8 wt.% dPLGA. Before application, the CPC containing (hPLGA or dPLGA) microspheres was created by adding 350 µl 2% $Na_2HPO_4$ in a 2-ml syringe (BD Plastipak, Becton Dickinson S.A., Madrid, Spain) with a closed tip and 30 s mixing using a mixing apparatus (Silamat, Vivadent, Schaan, Liechtenstein).

### Preparation of CPC-IP

CPC-IP was prepared as described by del Real *et al.*.[19] Shortly, 10 wt.% $NaHCO_3$ was added to the standard CPC powder. Before application, CPC-IP was created by adding 200 µl 2% $Na_2HPO_4$ to the powder mixture using a 2-ml syringe (BD Plastipak) with a closed tip and mixing these components for 15 s using a mixing apparatus (Silamat). The plunger was removed to add 150 µl 8% $NaH_2PO_4$ to decrease the pH necessary to produce $CO_2$ from $NaHCO_3$. The syringe was mixed for another 15 seconds.

### Morphological analysis

Microsphere size distribution was assessed by morphometrical analysis using a light microscope (Leica Microsystems AG, Wetzlar, Germany) and computer-based image analysis techniques (Leica® Qwin Pro-Image Analysis System, Wetzlar, Germany). Morphological appearance of both types of PLGA microspheres and pre-set CPCs (dimensions: Ø 7.8 mm; height 1.8 mm; created using a Teflon mold) was observed using scanning electron microscopy (SEM) (JEOL 6310 microscope at 10 kV). Additionally, interconnectivity and pore distribution were assessed for pre-set CPCs using microcomputed tomography (µCT). Pre-set CPCs (n=4) were digitally equal divided in a mid transversal direction into 2 volumes of interest (VOI), a VOI-top and VOI-bottom. Within those 2 volumes, macroporosity was determined and expressed as a percentage of the VOI by grey value selection. Samples were scanned at an energy of 100 kV and intensity of 98 µA with a resolution of 7.54 µm using an 1 mm aluminum filter (Skyscan-1072 X-ray microtomograph, TomoNT version 3N.5, Skyscan®, Belgium). Cone beam reconstruction (version 2.15, Skyscan®) was performed. µCT data were assessed using CT Analyser (version 1.4, Skyscan®). All scan and reconstruction parameters applied were identical for the three different CPC formulas.

**Setting time and Cohesion**

Initial and final setting time of CPCs was assessed using custom available Gillmore needles (ASTM C266). For this, a bronze block containing six holes (6 mm in diameter, 12 mm in height) was used as a mould and placed in a water bath at 37°C. Samples were mixed and injected into the mould in a retrograde fashion, after which the initial and final setting time was determined. Tests were done in fourfold. For the determination of cohesive properties, CPCs were injected in dd$H_2O$ at 37°C. During setting of CPCs it was inspected whether the CPCs retained their original configuration. Any anomalies were recorded.

**Porosity**

Total and macroporosity of pre-set samples was determined as described by Habraken *et al*..[26] Macroporosity is regarded as the porosity of CPCs, in which the pores are generated by the (i) degradation of PLGA microspheres, or (ii) entrapped $CO_2$ bubbles. Total porosity is the macroporosity plus the intrinsic microporosity of the cement. For the determination of porosity characteristics, CPC-IP and both CPC-PLGA pre-set samples of known volume were placed in an oven at 650 °C for 2 h (n=4). Subsequently, total porosity and macroporosity could be calculated using mass determinations of pre-set CPCs following equations described by Habraken *et al*..[26]

***In vitro* degradation studies**

For the *in vitro* degradation studies, pre-set CPC-IP and both types of CPC-PLGA discs were incubated in 1.5 ml phosphate-buffered saline (PBS) at 37 °C on a shaking apparatus for 12 weeks (*n*=4) with refreshment of the incubation medium at 1, 2, 3, 4, 5, 6, 9 and 12 weeks. At these time points, CPCs were retrieved for morphological analysis (SEM) and samples (volume: 200 ul) were taken from the incubation media for analysis of pH (PHM210, Standard pH meter, MeterLab™ Radiometer, Copenhagen), calcium amount and PLGA-degradation products. After each incubation medium sampling, the volume of the incubation medium was increased to 1.5 ml by adding 200 ul fresh PBS. All results were corrected for dilution of the incubation media over time.

The absolute amounts of $Ca^{2+}$ in the incubation media were analyzed by the orthocresolphtalein complexone (OCPC) method (Sigma-Aldrich Chemie B.V., Zwijndrecht, the Netherlands) as a measure of CPC mass degradation. In brief, 0.5 M acetic acid was added to the incubation medium sample. After overnight incubation on a shaking apparatus, 300 µl working solution was added to 10 µl sample in a 96-wells plate. Working solution consisted of (a) OCPC solution (80 mg OCPC in 75 ml milliQ + 0.5 ml 1 M KOH + 0.5 ml 0.5 N acetic acid), (b) 14.8 M ethanolamine/boric acid buffer (pH = 11), (c) 8-hydroxyquinoline (1 g in 20 ml 95% ethanol), and milliQ, in a ration of 5:5:2:88 (a,b,c,d). A standard curve was

generated by preparing serial dilutions of $CaCl_2$ (0-100 µg/ml).

The amounts of glycolic and lactic acid in the incubation media were analyzed by reverse-phase high performance liquid chromatography (RP-HPLC) as a measure of PLGA degradation. The system consisted of a Hitachi L2130 HPLC pump, a Hitachi L-2400UV detector, a Hitachi L-2200 autosampler and an Atlantis dC18 column (Waters), 250 mm x 4.6 mm, 5 µm. Two mobile phases were used: 1% acetonitrile in 20 mM $NaH_2PO_4$, pH 2.2 (mobile phase A), and 100% acetonitrile (mobile phase B). The flow was 1 ml $min^{-1}$, the injection volume was 20 µl and the ultraviolet detection wavelength was 210 nm.

**Design of the *in vivo* experiment**

A total of 12 adult male Wistar rats with an average weight of 275 g were used, observing national guidelines for the care and use of laboratory animals. The research protocol was approved by the Experimental Animal Committee of the Radboud University, Nijmegen, The Netherlands (DEC2010-027). A total of 24 augmentation sites (i.e. 8 for CPC-IP, 8 for CPC-hPLGA and 8 for CPC-dPLGA) were randomly distributed over the 12 animals, which each received two different CPCs injected within a PTFE ring on the parietal bone.

The animals were pre-medicated by an intramuscular injection of fentanyl (2.7 ml/kg) to reduce operative pain. General anesthesia was initiated by 4% isoflurane. Subsequently, rats were placed in a ventral position, intubated, and connected to an inhalation ventilator with a constant volume of a mixture of 2% isoflurane, 0.4% $N_2O$ and 0.4% $O_2$. The skull was shaved and disinfected with povidone-iodine. Lidocaine HCL 1%/epinephrine was subcutaneously injected to minimize pain and reduce bleeding. A longitudinal incision was made from the nasal bone to the occipital protuberance to visualize the parietal periosteum. Subsequently, the periosteum was undermined and the skin flap was reflected, exposing the parietal bone. A hollow trephine drill (outer diameter: 7 mm, inner diameter 6 mm; ACE Dental Systems, Brockton, MA, USA) in a dental hand piece (WS-75E/KM, W&H Dentalwerk Burmoos GmbH, Austria) was used to drill two circular slits in the parietal skull. Drilling was performed only in the upper part of the cortical plate to create a circular slit with a depth of approximately 0.5mm. The bony surface was cleaned with a gauze. PTFE rings were positioned into the slits and checked on their immobility. Hereafter, CPCs were injected inside the rings (n=8 for each implantation material). In this way, implants in the shape of discs were created, with a diameter of 6 mm and a height of ~2 mm augmented on top of the parietal bone (Figure 1a,b). After the initial setting of CPC, the overlying skin flaps were closed in separate layers. Buprenorphine (150 µg/kg) was applied for three days to reduce postoperative pain.

### Histological processing

Animals were sacrificed after a 12-week implantation period using an overdose of $CO_2$. Implants with surrounding tissue were retrieved and fixed in 10% formalin for 36 h. Specimens were dehydrated in graded series of alcohol and decalcified in EDTA for 12 h. Before embedding in paraffin, specimens were cut exactly transversally (i.e. through the middle of the augmented area) to warrant preparation of microtome sections within the area of interest. Intermittent coronal microtome sections (5 µm) were prepared as shown in Figure 1a, and stained with hematoxylin-eosin (HE). Light microscopic evaluation of at least eight sections per specimen was done using an optical microscope (Leica Microsystems AG, Wetzlar, Germany) consisting of a complete morphological description.

### Histomorphometry

Bone formation was quantified using computer-based image analysis techniques (Leica Qwin Pro-image analysis system) of at least four histological sections (HE-stain) per specimen. The region of interest (ROI) consisted of the augmented area inside the ring. This area was bordered by the original parietal bone surface and the PTFE bars with a height of 2 mm (Figure 1a). To determine the amount of bone apposition, newly formed bone was marked manually based on pixel value detection and expressed as a percentage of the ROI. Furthermore, the maximum height of bone ingrowth (i.e. the longest perpendicular distance between the original parietal bone surface and newly formed bone within the ROI) was measured for all histological sections.

### Statistical Analysis

Data are presented as mean ± standard deviation. Analysis of variance (ANOVA) with posthoc Tukey-Kramer Multiple Comparisons Tests were performed on data obtained from the Gillmore test, *in vitro* degradation studies and histomorphometric analysis. For all statistics, GraphPad InStat (GraphPad Software, San Diego, CA, USA) was used. Differences were considered to be significant at $p<0.05$.

| *Setting (min)* | *CPC-IP* | *CPC-hPLGA* | *CPC-dPLGA* |
|---|---|---|---|
| Initial setting time | 3.7 ± 0.2 | 4.5 ± 0.3 | 4.5 ± 0.3 |
| Final setting time | 17.5 ± 0.5 | 11.6 ± 0.5 | 12.4 ± 0.4 |
| *Porosity (%)* | | | |
| Calculated total porosity | 76.6 ± 0.2 | 72.2 ± 1.0 | 70.9 ± 0.1 |
| Calculated microporosity | 23.4 ± 0.2 | 27.7 ± 1.0 | 28.9 ± 0.1 |
| Calculated macroporosity | 53.2 ± 0.4 | 44.5 ± 1.9 | 42.0 ± 0.2 |
| µCT porosity distribution VOI-top : VOI-bottom | ~57:43 | ~50:50 | ~50:50 |

Table 1: Material characteristics. Setting time and porosity.

# RESULTS

### Material characteristics: morphological analysis, handling and porosity

Morphological analysis on the size and distribution of pores in CPC-IP created by $CO_2$ foaming demonstrated lack of pore uniformity, irregular shape (Figure 2c,f), and pore sizes ranging from 0.1 µm up to 500 µm. The hPLGA and dPLGA microspheres showed a spherical shape with a size of 33.4±7.4 µm and 39.8±3.9 µm, respectively. However, the surface of dPLGA microspheres was less smooth and irregular compared to the surface of hPLGA microspheres (Figure 2a,b). CPC-hPLGA and CPC-dPLGA demonstrated to contain microspheres that maintained their integrity and shape (Figure 2d,e). Besides macroporosity, as created by $CO_2$ bubbles and PLGA microspheres, all CPCs showed also a characteristics additional intrinsic microporosity (Figure 2c,d,e).

Results of the Gillmore test assessing the setting time of the CPC composites are listed in Table 1. CPC-hPLGA and CPC-dPLGA showed similar initial setting times of approximately 4.5 minutes. Initial setting of CPC-IP was significantly faster in 3.7 minutes ($p<0.05$). However, final setting time of CPC-IP (17.5 minutes) was significantly slower compared to the final setting times of both CPC-hPLGA and CPC-dPLGA (~10 minutes; $p<0.001$). Cohesion tests revealed no disintegration for any of the CPCs upon injection in an aqueous environment during setting.

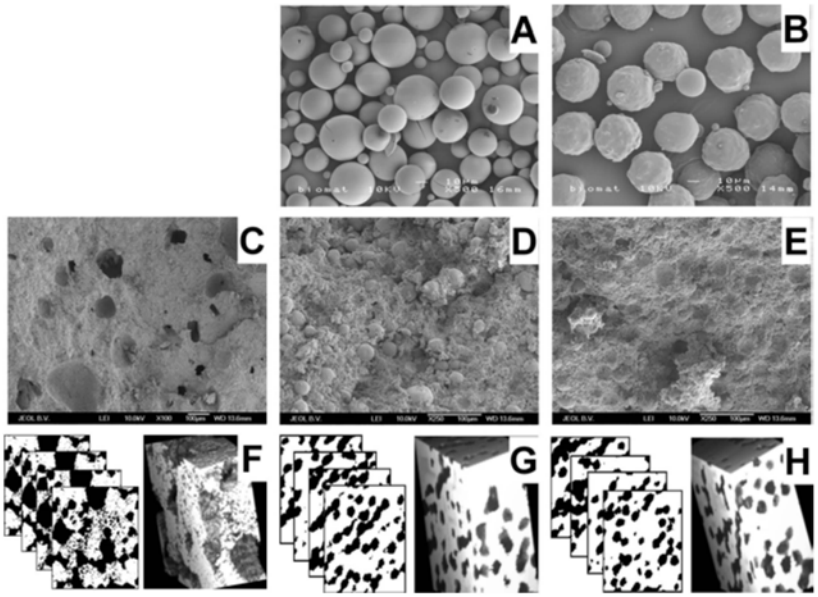


Figure 2: Material morphology. Morphological analysis of materials. (a-e) Scanning electron microscopic evaluation of (a) hollow PLGA microspheres, (b) dense PLGA microspheres, (c) pre-set CPC-IP, (d) pre-set CPC-hPLGA , and (e) pre-set CPC-dPLGA. (f-h) Representation of four binary sagittal sections (left) subtracted from the 3D model obtained by µCT (right) of (f) CPC-IP, (g) CPC-hPLGA and (h) CPC-dPLGA (CPC is represented in white; created porosity is indicated in black).

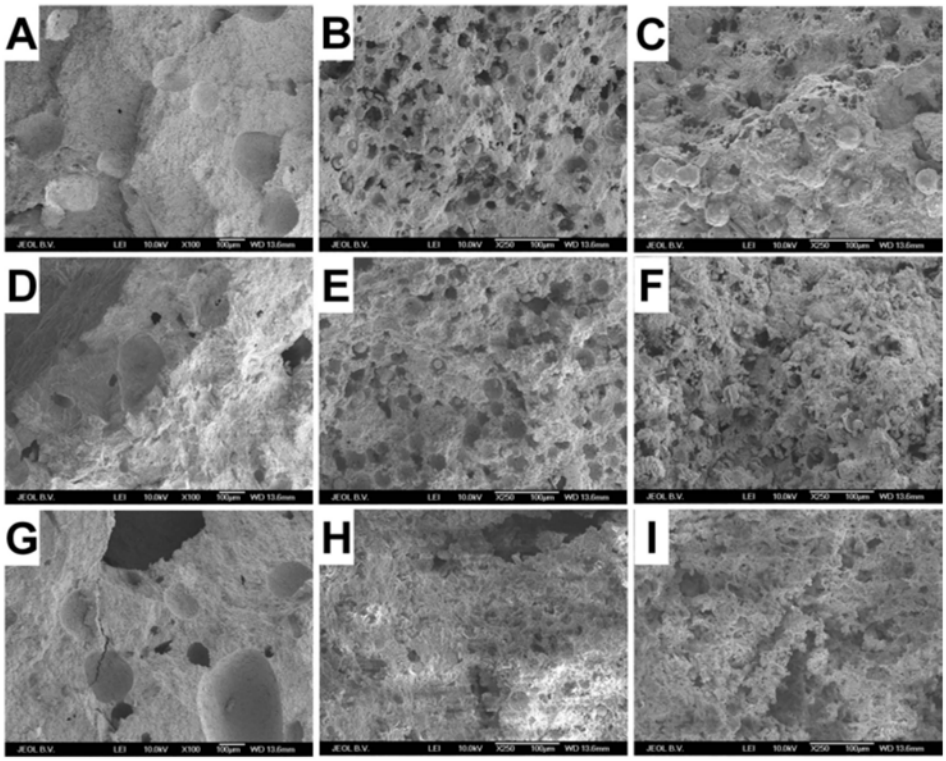


Figure 3: Scanning electron microscopic imaging of CPC degradation. SEM micrographs of CPC-IP (a,d,g), CPC-hPLGA (b,e,h) and CPC-dPLGA (c,f,i) after 2 (a-c), 4 (d-f) and 12 (g-i) weeks of incubation, respectively.

The total porosity, originating from the micro- and macroporosity calculations, of all three CPCs is presented in Table 1. The macroporosity in CPC-hPLGA and CPC-dPLGA was 44.5±1.9% and 42.0±0.2%, respectively. The macroporosity of the CPC-IP was 53.2±0.4%.

µCT analysis of CPC-hPLGA and CPC-dPLGA samples showed a homogenous distribution of porosity throughout the specimens, as presented by an equal porosity measured in the top and bottom volumes of the pre-set CPCs (Table 1). In contrast, CPC-IP samples showed a higher porosity in the top volume compared to the bottom volume (57±% vs. 43±%; $p<0.001$; Table 1). µCT sections demonstrated substantial interconnectivity of micro- and macropores in all CPCs (Figure 2f,g,h).

### *In vitro* degradation studies

SEM images of the CPCs after 2, 4 and 12 weeks of incubation time are shown in Figure 3. No obvious CPC-matrix degradation was observed for CPC-IP at any time point (Figure 3a,d,g). Both CPCs containing PLGA microspheres showed initial microsphere degradation after 2 weeks of incubation (Figure 3b,c). Remnants of shells of hPLGA microspheres were observed within the CPC-matrix (Figure 3b). In contrast, the initial internal compact structure of dPLGA microspheres became porous, indicating an intermediate stage in the degradation process (Figure 3c). After 4 weeks, both types of microspheres had degraded to a large extent (Figure 3e,f). After 12 weeks of incubation, no PLGA remnants were visible in PLGA containing CPCs and the CPC-matrix partially lost its structure after retrieval. In contrast, the macroporous structure of CPC-IP was still maintained (Figure 3h,i).

CPC-IP showed a gradual decrease in pH of the incubation media in the first 4 weeks, after which pH stabilized at 6.3±0.4 after 6 weeks (Figure 4a). pH measurements of delayed porous CPC showed already after 2 weeks of incubation a significant larger decrease in pH to 4.4±0.3 and 3.4±0.1 for CPC-hPLGA and CPC-dPLGA, respectively ($p<0.001$). Furthermore, CPC-dPLGA showed a significantly lower pH at all time points compared to CPC-hPLGA ($p<0.001$). These results revealed a higher release of acidic monomers in the incubation media using CPC-dPLGA.

The cumulative CPC-matrix degradation determined by $Ca^{2+}$-release in the incubation media for CPC-IP was limited and increased from around 20 to 130 µg $Ca^{2+}$/g CPC-matrix at 12 weeks (Figure 4b). In contrast, both PLGA containing CPCs showed substantial degradation of the CPC-matrix according to a significant increase of $Ca^{2+}$ into the incubation media ($p<0.001$) over time to reach ~5000 and 11000 µg $Ca^{2+}$/g CPC–matrix for CPC-hPLGA and CPC-dPLGA, respectively. Moreover, CPC-dPLGA showed a significant higher CPC-matrix degradation compared to CPC-hPLGA at all time points ($p<0.001$; Figure 4b).

Both acidic monomers were released at a comparable rate from CPC-hPLGA and CPC-dPLGA and the glycolic acid release data are shown in Figure 4c. The data show a gradual increase in concentration of glycolic acid in the incubation media from 2 to 12 weeks, after which the release reaches 100% for both CPC-hPLGA and CPC-dPLGA. Significantly accelerated release of glycolic acid ($p<0.001$) was observed for CPC-dPLGA compared to CPC-hPLGA in the period between 3 to 9 weeks of incubation time.

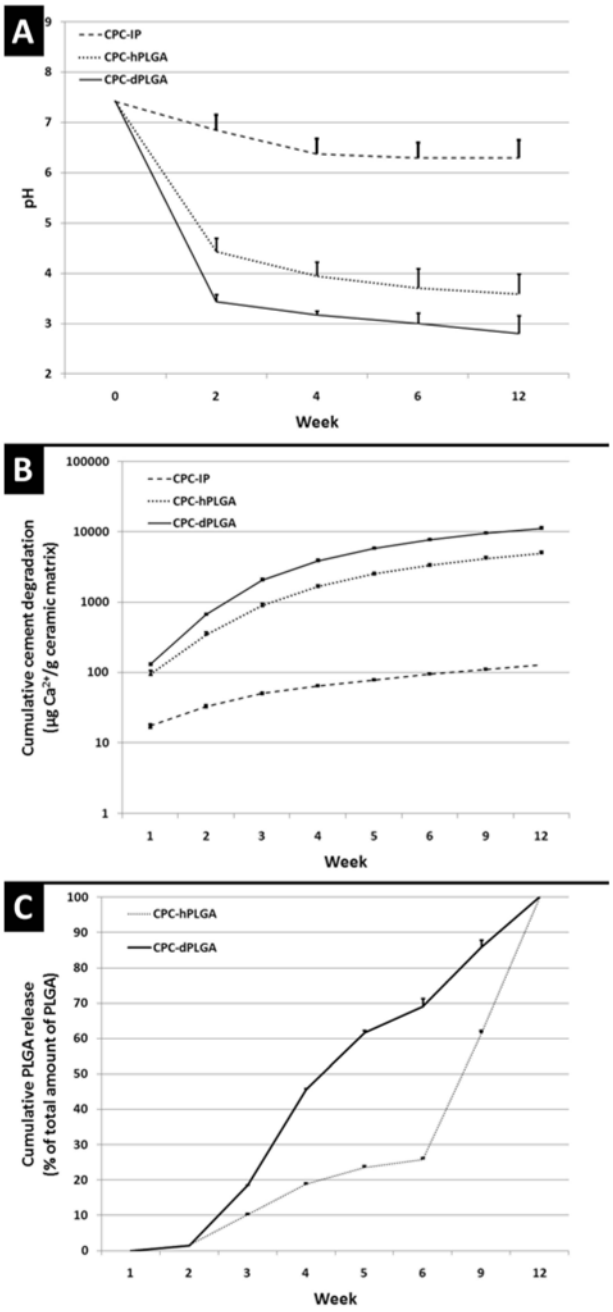


Figure 4: In vitro degradation studies. (a) pH measurements of the incubation media after CPC degradation. (b) Absolute amount (ug) of dissolved $Ca^{2+}$/ gram CPC-matrix. (c) Glycolic acid detection by RP-HPLC. Significant differences in time and between material groups: CPC-IP, CPC-hPLGA and CPC-dPLGA were found in pH and dissolved $Ca^{2+}$ measurements. RP-HPLC results showed significant differences between CPC-dPLGA and CPC-hPLGA in the period from 3 to 9 weeks of incubation.

### *In* vivo follow-up and sample retrieval

The number of augmentation sites per group that were created, retrieved, and used for analysis are presented in Table 2. One animal died during surgery as a result of skull bone penetration, followed by intracranial bleeding. The 12 week follow-up period was uneventful for the remaining 11 animals. At sacrifice, a total of 22 augmentation areas were retrieved, of which macroscopic observation showed rigidly immobilized PTFE rings in which residual CPC material was present and overgrown with (layers of) soft tissue. During histological processing, one specimen was lost.

### Descriptive histology

In the histological sections, PTFE borders and residual CPC-matrix within the ring were lost during decalcification, sectioning and staining of the histological sections. Nevertheless, the location of the PTFE borders could be clearly identified and no adverse tissue reaction or inflammatory response to the PTFE could be observed.

All experimental groups displayed new bone formation originating from the skull bone both centrally and at the borders of the PTFE ring boundaries (Figure 5a,b,c). No bone formation was seen originating from the soft tissue layer above the implants. Between CPCs and newly formed bone direct contact could be observed. CPC-IP showed bone and soft tissue ingrowth into the macropores. Augmentation with CPCs containing hPLGA or dPLGA microspheres showed limited bone formation and soft tissue ingrowth. The morphological appearance of newly formed bone showed similarity with the pore sizes and shapes (Figure 5a,b,c). Occasionally, at the outside the borders of the PTFE ring, limited bone formation was observed extending from the existing bony surface (Figure 5b).

At a higher magnification, newly formed bone appeared as vital bone tissue containing osteoblasts, osteoid, osteocytes and blood vessels in all CPCs (Figure 6a,b). Furthermore, soft tissue was apparent between bone voids including blood vessels and osteoblasts lining the surface (Figure 6a,b). In addition, multinucleated cells were found at the boundaries of the remaining CPC (Figure 6c).

### Histomorphometry

The amount of newly formed bone was significantly higher in the CPC-IP group (10.8±1.8%) compared to both PLGA containing CPCs (CPC-hPLGA: 7.0±2.4%, CPC-dPLGA: 6.8±2.6%; $p<0.05$; Figure 7). The maximum augmented bone height (Figure 8) was significantly higher for CPC-IP (947±175 µm) compared to CPC-dPLGA (364±178 µm; $p<0.05$), but not in comparison to CPC-hPLGA (601±493 µm). No significant differences were observed between CPC-hPLGA and CPC-dPLGA for either bone area or appositional bone height.

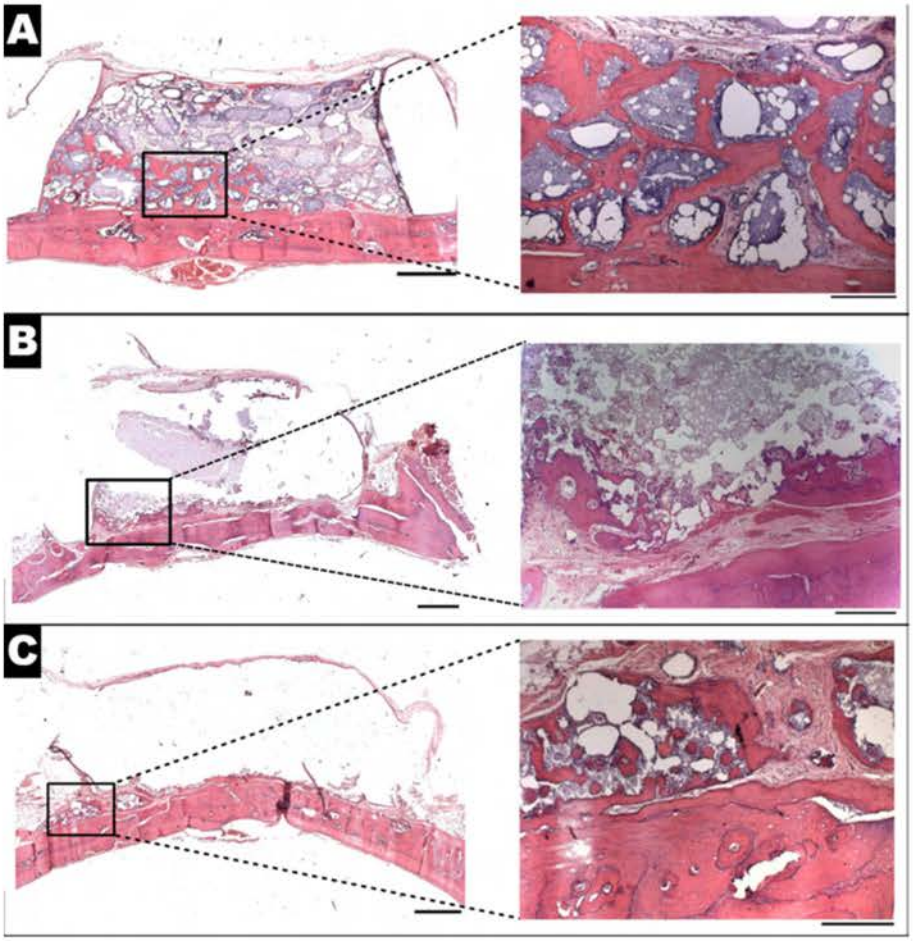


Figure 5: Histology. Hematoxylin-eosin staining of coronal histological sections after 12 weeks. PTFE borders together with some residual CPC contained by the ring was lost during sectioning and staining of the histological sections. (a-c) Whole images of the representative slices of the three groups: CPC-IP, CPC-hPLGA, CPC-dPLGA respectively (bar = 1 mm). At the right side, a higher magnification of the selected square from the three groups: CPC-IP, CPC-hPLGA, CPC-dPLGA respectively (bar = 250 µm). At a higher magnification, note the similarity of the morphology of newly formed bone showed with the pore sizes and shapes.

| Group | No. of implants placed | No. of implants retrieved after 12 weeks | No. of implants used for histomorphometric analysis |
|---|---|---|---|
| CPC-hPLGA | 8 | 7 | 6 |
| CPC-dPLGA | 8 | 7 | 7 |
| CPC-IP | 8 | 8 | 8 |

Table 2: Sample overview. Number of samples placed, retrieved and used for histological and histomorphometric analysis per experimental group (deviation from number of samples retrieved due to dead of 1 rat and histological processing).

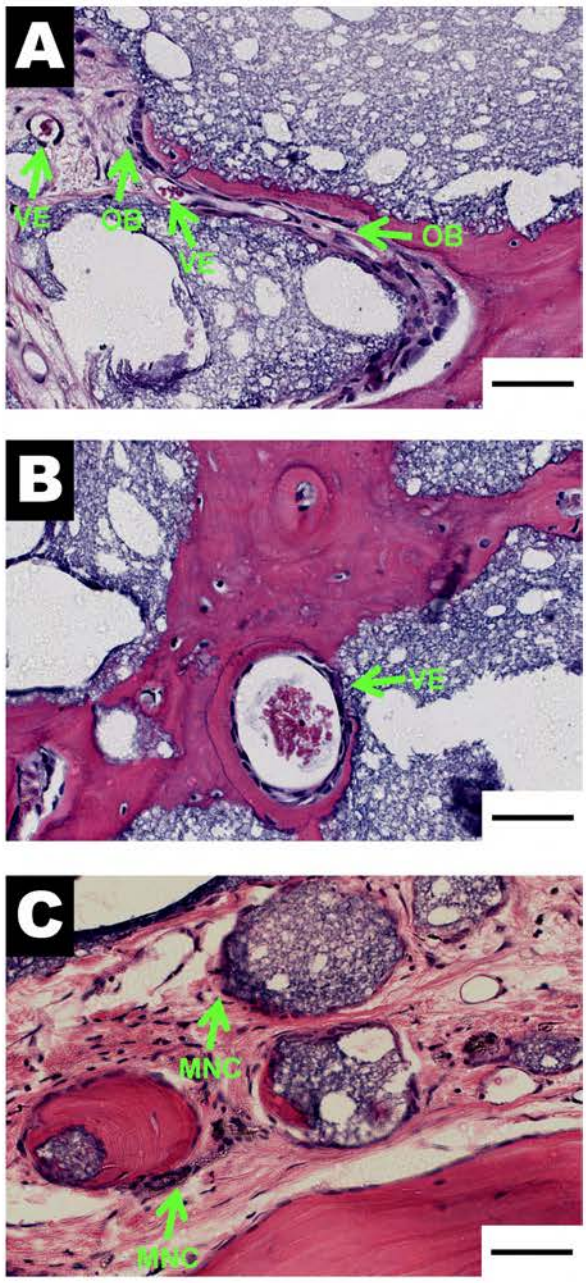


Figure 6: Histology. High magnification of hematoxylin-eosin staining of coronal histological sections after 12 weeks. (a-b) Newly formed bone in close contact to remaining CPC appeared as vital bone tissue containing lining osteoblasts (OB), osteoid, osteocytes and blood vessels (VE). (c) Multinucleated cells (MNC) at the boundaries of remaining CPC. (bar = 50 µm).

# DISCUSSION

The aim of the present study was to compare the *in vitro* degradation and *in vivo* bone conductive capacity of CPCs that follow fundamentally different strategies to become porous. Instantaneous porosity was obtained by the formation and entrapment of gas bubbles during CPC setting, while incorporation of PLGA microparticles into CPC was used to obtain a delayed porosity after PLGA degradation. An additional aspect was the use of two different types of PLGA microparticles (i.e. hollow vs. dense), for which it was hypothesized that an increased amount of acidic by-products might improve CPC degradation and hence create more space for bone formation.

Instantaneously porous CPC directly allows penetration of (body) fluids and ingrowth of cells, whereas the main drawback has been described as the lack of control on pore size and distribution.[19;20;28] Morphological analysis of instantaneously created pores in the present study confirmed this irregularity in size and distribution, of which the latter is related to the upward migration of gas bubbles during setting. It is likely that this effect becomes even more apparent with increasing dimensions of the material. Alternatively, delayed porosity within CPC after PLGA microsphere degradation allows excellent control of pore sizes and a gradual replacement of CPC by newly formed bone. Additionally, it is possible to induce a slow and constant release of biological factors incorporated in PLGA microspheres.[23] Nevertheless, the main drawback of the inclusion of PLGA microspheres within CPC is the delay in the creation of porosity and this process depends largely on the degradation characteristics of the used PLGA.[24;26;29;30] In this study, one type of PLGA was used to produce hollow and dense microspheres. Morphological analysis confirmed that incorporation of either of these microspheres results in a homogeneous distribution of microspheres throughout the CPC-matrix.

*In vitro*, CPC-matrix degradation was observed morphologically by SEM and chemically by $Ca^{2+}$ and PLGA-degradation product measurements in the incubation media. Limited instantaneously porous CPC degradation was observed, while CPCs containing PLGA microspheres showed morphological and chemical dissolution in time. This enhanced passive degradation was most likely caused by the release of acidic by-products from PLGA hydrolysis and subsequent decrease of pH in the incubation media. In line with these findings, CPC with dense PLGA microparticles degraded significantly more than CPC with hollow PLGA microspheres. Although the volumetric amount of PLGA microspheres was similar for CPC incorporating hollow or dense PLGA microspheres, the difference in PLGA mass (i.e. ~2-fold higher for dense PLGA microspheres) resulted in an increased amount of acidic by-products in the incubation media. The associated

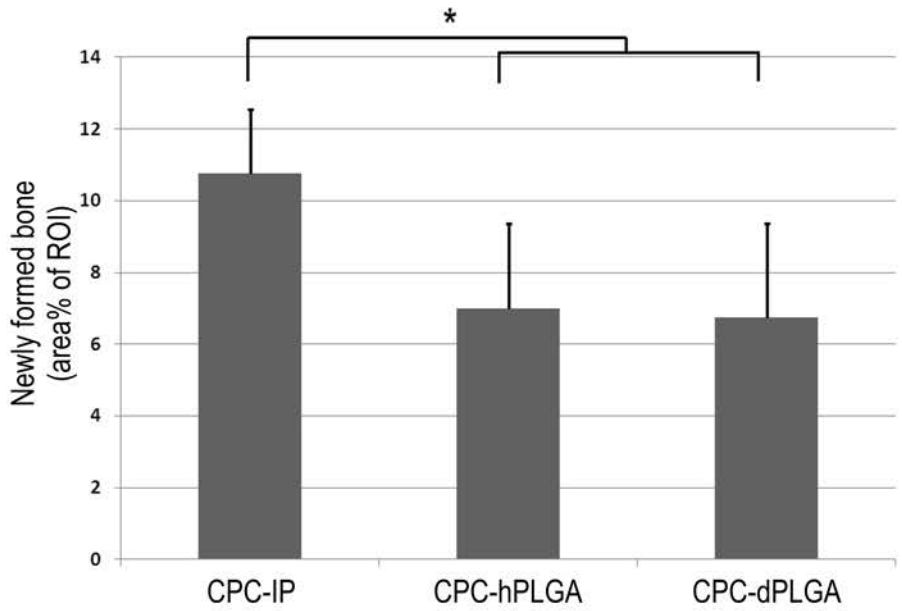


Figure 7: Histomorphometry. Newly formed bone (%) within the region of interest (mean ± SD) for CPC-IP, CPC-hPLGA and CPC-dPLGA after 12 weeks (* indicates significant difference; $p<0.05$).

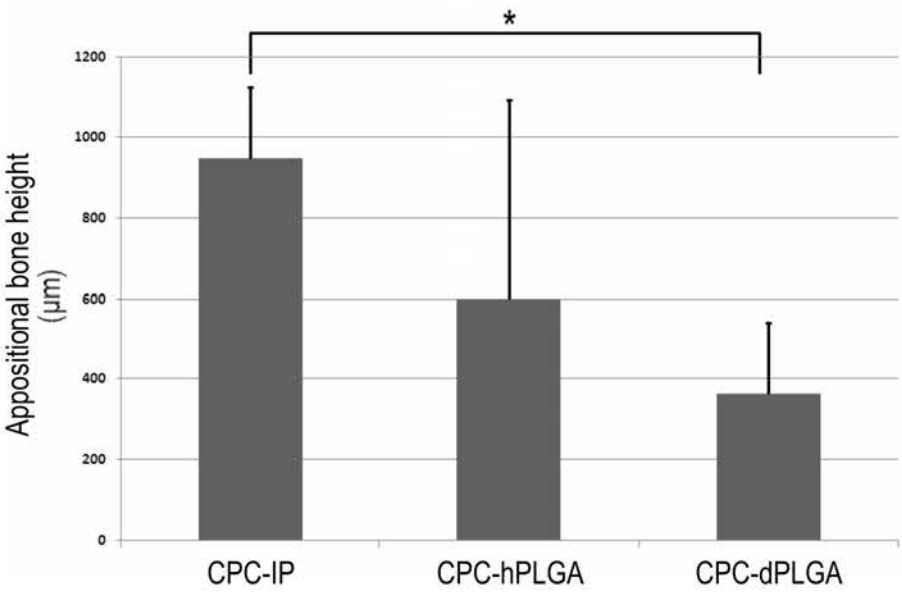


Figure 8: Histomorphometry. Appositional bone height (mean ± SD) for CPC-IP, CPC-hPLGA and CPC -dPLGA after 12 weeks of implantation time ($^{x}$ indicates significant difference; $p<0.05$).

pH decrease can have an accelerating effect on the degradation of PLGA microspheres and CPC-matrix.[29;31] This accelerated degradation is the result of an autocatalytic effect due to the low pH of the surrounding environment generated by the previously released acidic by-products. The presence of an earlier and higher concentration of acidic by-products in the incubation media for CPC containing dense microspheres confirmed the occurrence of such an autocatalytic effect. Resulting from the above-mentioned acidification of the environment, the creation of porosity was enhanced by CPC dissolution in the direct vicinity of dense microspheres. Thus, while porosity was already present after injection of instantaneously porous CPC, gradual formation of pores or further *in vitro* CPC-matrix dissolution was only observed for CPCs containing PLGA microspheres (dense > hollow).

Several preclinical augmentation models have been developed in various animals to evaluate bone substitute materials.[32] However, in smaller animals, bone augmentation surgery is limited to flat bone surfaces such as the mandible or parietal bone. Two of the most relevant parameters that influence the outcome of onlay grafts are rigid immobilization and a high degree of graft contact to the recipient surface.[33;34] Earlier studies described the use of titanium domes or rings with or without screws to ensure immobilization.[35-37] This study showed that also PTFE rings can be successfully fixed to the parietal bone by a circular drilled slit allowing rigid immobilization. Furthermore, CPCs completely filled the PTFE ring and were already initially set on the bony surface after three to four minutes resulting in a high degree of graft contact. Nevertheless, sufficient time was available for handling in order to achieve precise adjustment to the augmentation site within the PTFE ring. Thus, CPCs showed an uncomplicated injection and setting times within a clinical acceptable range. Furthermore, the *in vivo* model allowed rigid immobilization of these CPCs within a PTFE barrier for biological or even radiographic evaluation.

In this study the bone conductive capacity of CPCs with an instantaneously versus delayed porosity were compared. The *in vivo* part of the study showed bone apposition reaching volumetric amounts of only up to 10% of the augmentation area and a maximum augmentation height of ~1 mm after 12 weeks of implantation. Histomorphometric analysis demonstrated that the use of instantaneously porous CPC resulted in the significantly largest amount of bone formation and the highest augmentation height compared to delayed porous CPCs. Although a significant difference was observed in the *in vitro* part, no statistically significant differences in bone apposition were found between CPCs with dense or hollow PLGA microspheres *in vivo*.

Although no temporal information was obtained *in vivo* in the present study, it is likely that the instantaneously porous CPC allowed direct influx of body fluid,

proteins and cells into the porous CPC after application. This advantage resulted in a jump-start in bone formation compared to CPCs with a delayed porosity. Ruhe *et al.* confirmed by fluorochrome labeling that bone formation started at the cement surface of instantaneously porous CPC and proceeded into the macropores already after 1 week after implantation.[22] In contrast, the possibility of tissue ingrowth in delayed porous CPC increases gradually during implantation time with PLGA degradation.[38] Previous reports have shown that the amount of PLGA in CPC containing hollow PLGA microspheres decreased ~3.5-fold during 4 weeks after *in vivo* implantation, resulting in a porous structure to allow tissue ingrowth.[38] However, such information was not yet available for CPC containing dense PLGA microspheres.

While most of the delayed porous CPC was lost during histological processing, the CPC-matrix of instantaneously porous CPC remained in place and was infiltrated for the major part of the porosity with soft tissue. Only limited ingrowth of soft and hard tissue was observed in delayed porous CPC. In view of these findings, pore size dimension and interconnectivity of porosity has been described as a main prerequisite for tissue ingrowth.[14;39;40] In a recent review of Bohner, it was concluded that the diameter and pore interconnections in CPC must be larger than 50 μm to allow fluid flow and cell penetration.[13] Instantaneously porous CPC allowed the formation of porosity that equals (or exceeds) these dimensions. Moreover, previous reports described a pore size distribution of instantaneously porous CPC in the range 0.003-300 μm.[20] Furthermore, Ruhe *et al.* concluded that the pores of instantaneously porous CPC could be regarded as completely interconnected.[22] Quite the opposite accounts for delayed porous CPC, which has a theoretical pore size of only around 40 μm after PLGA hydrolysis. A contributing factor is that, as a consequence of the standardized production process of dense PLGA microspheres, their size distribution becomes significantly smaller compared to that of hollow equivalents. In view of this, Habraken *et al.* concluded that microspheres of different sizes can create a closer packing in which little spheres serve as interstitials between the larger ones inducing a higher CPC porosity.[26] Nevertheless, an (potential) open porous structure was observed by SEM and μCT for all three types of CPC. In contrast with the recent findings, previous *in vivo* studies indicated that hollow PLGA microspheres of even 20 μm appeared large enough for bone ingrowth.[24;41]

In view of the above mentioned, we suppose that the lack of bone and soft tissue ingrowth into both dPLGA hPLGA CPC can be due to: (1) a difference between the *in vitro* and *in vivo* degradation behavior of PLGA particles resulting in a delayed *in vivo* degradation, (2) a negative effect of the acid released from dense PLGA microspheres on the biological process of new bone formation, or (3) the buffering capacity of the body, which diminishes the effects of acid

release to induce porosity by PLGA degradation. This latter effect can perhaps be enhanced by the used implantation sites where the CPC is mainly surrounded and in contact with well-vascularized soft tissue instead of bony tissue as in condylar defects. Currently, no final answer can be given, which of these three explanations is most valid. Therefore, further research is necessary to elucidate the effect of the temporal degradation of PLGA microspheres incorporated in CPC (and PLGA degradation products) on the biological performance *in vivo* in bone augmentation models.

While previous CPC studies using critical size defects showed substantial bone formation, bone augmentation procedures rely on the complex biological process of appositional bone growth. In view of this, only a limited amount of bone formation was observed using either instantaneously or both delayed porous CPC in this study. The bone implant contact surface is limited to the surface and a longer implantation time might be required to achieve complete filling of the porosity within CPCs by bone. Enhancement of this process is possible by the inclusion of growth factors stimulating bone growth (such as bone morphogenetic proteins). In view of this, delayed porous CPC has the advantage of adsorption and release of growth factors from PLGA microspheres incorporated into CPC compared to instantaneously CPC. Therefore, further research is necessary to enhance the amount and bone apposition speed of CPCs for complicated bone augmentation procedures.

## CONCLUSION

This study compared CPCs for bone substitution with instantaneous porosity generated by $CO_2$ bubbles or delayed porosity generated by incorporation of PLGA microspheres (either hollow or dense). All CPCs showed appropriate clinical handling and an interconnected porous structure with a final total porosity above 70% (v/v). In vitro degradation studies showed direct porosity in instantaneously porous CPC. However, gradual *in vitro* formation of pores and further *in vitro* CPC-matrix dissolution was only observed for CPCs containing PLGA microspheres. *In vivo* data demonstrated limited bone formation reaching only up to 10% of the augmented area. A significantly higher amount of appositional bone formation in combination with a higher augmentation height for augmentation with instantaneously porous CPC was found compared to delayed porous CPC. No significant differences in bone apposition were observed between the incorporation of hollow or dense PLGA microspheres into CPC. Further research is necessary to enhance the amount and bone apposition speed of CPCs for bone augmentation procedures before used in a clinical setting.

# REFERENCES


1. McAllister B.S. and Haghighat K. Bone augmentation techniques. Journal of Periodontology 78, 377, 2007.
2. Nevins M., Mellonig J.T., Clem D.S., Reiser G.M., and Buser D.A. Implants in regenerated bone: long-term survival. International Journal of Periodontics & Restorative Dentistry 18, 34, 1998.
3. McAllister B.S. and Gaffaney T.E. Distraction osteogenesis for vertical bone augmentation prior to oral implant reconstruction. Periodontology 2000 33, 54, 2003.
4. Tamimi F., Torres J., Gbureck U., Lopez-Cabarcos E., Bassett D.C., Alkhraisat M.H., and Barralet J.E. Craniofacial vertical bone augmentation: a comparison between 3D printed monolithic monetite blocks and autologous onlay grafts in the rabbit. Biomaterials 30, 6318, 2009.
5. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. Sinus floor augmentation surgery using autologous bone grafts from various donor sites: a meta-analysis of the total bone volume. Tissue Engineering Part B Reviews 16, 295, 2010.
6. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. A Meta-Analysis of Histomorphometric Results and Graft Healing Time of Various Biomaterials Compared to Autologous Bone Used as Sinus Floor Augmentation Material in Humans. Tissue Engineering Part B Reviews 16, 493, 2010.
7. Nkenke E. and Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clinical Oral Implants Research 20 Suppl 4, 124, 2009.
8. Wang S., Zhang Z., Zhao J., Zhang X., Sun X., Xia L., Chang Q., Ye D., and Jiang X. Vertical alveolar ridge augmentation with beta-tricalcium phosphate and autologous osteoblasts in canine mandible. Biomaterials 30, 2489, 2009.
9. Busenlechner D., Tangl S., Mair B., Fugger G., Gruber R., Redl H., and Watzek G. Simultaneous in vivo comparison of bone substitutes in a guided bone regeneration model. Biomaterials 29, 3195, 2008.
10. Merkx M.A., Maltha J.C., and Stoelinga P.J. Assessment of the value of anorganic bone additives in sinus floor augmentation: a review of clinical reports. International Journal of Oral and Maxillofacial Surgery 32, 1, 2003.
11. Katranji A., Fotek P., and Wang H.L. Sinus augmentation complications: etiology and treatment. Implant Dentistry 17, 339, 2008.
12. Brown, W.E. and Chow, L.C. A new calcium phosphate setting cement. Journal of Dental Research 62, 682, 1983.
13. Bohner M. Design of ceramic-based cements and putties for bone graft substitution. European Cells & Materials Journal 20, 1, 2010.
14. Karageorgiou V. and Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 26, 5474, 2005.
15. LeGeros R.Z. Biodegradation and bioresorption of calcium phosphate ceramics. Clinical Materials 14, 65, 1993.
16. Gomi K., Lowenberg B., Shapiro G., and Davies J.E. Resorption of sintered synthetic hydroxyapatite by osteoclasts in vitro. Biomaterials 14, 91, 1993.
17. Schilling A.F., Linhart W., Filke S., Gebauer M., Schinke T., Rueger J.M., and Amling M. Resorbability of bone substitute biomaterials by human osteoclasts. Biomaterials 25, 3963, 2004.
18. Ooms E.M., Wolke J.G., van de Heuvel M.T., Jeschke B., and Jansen J.A. Histological evaluation of the bone response to calcium phosphate cement implanted in cortical bone. Biomaterials 24, 989, 2003.
19. del Real R.P., Wolke J.G., Vallet-Regi M., and Jansen J.A. A new method to produce macropores in calcium phosphate cements. Biomaterials 23, 3673, 2002.
20. del Real R.P., Ooms E., Wolke J.G., Vallet-Regi M., and Jansen J.A. In vivo bone response to porous calcium phosphate cement. Journal of Biomedical Materials Research A 65, 30, 2003.
21. Link D.P., van den Dolder J., Jurgens W.J., Wolke J.G., and Jansen J.A. Mechanical evaluation of

implanted calcium phosphate cement incorporated with PLGA microparticles. Biomaterials 27, 4941, 2006.

22. Ruhe P.Q., Kroese-Deutman H.C., Wolke J.G., Spauwen P.H., and Jansen J.A. Bone inductive properties of rhBMP-2 loaded porous calcium phosphate cement implants in cranial defects in rabbits. Biomaterials 25, 2123, 2004.
23. Plachokova A., Link D., van den Dolder J., van den Beucken J., and Jansen J. Bone regenerative properties of injectable PGLA-CaP composite with TGF-beta1 in a rat augmentation model. Journal of Tissue Engineering and Regenerative Medicine 1, 457, 2007.
24. Bodde E.W., Habraken W.J., Mikos A.G., Spauwen P.H., and Jansen J.A. Effect of polymer molecular weight on the bone biological activity of biodegradable polymer/calcium phosphate cement composites. Tissue Engineering Part A 15, 3183, 2009.
25. Ruhe P.Q., Hedberg E.L., Padron N.T., Spauwen P.H., Jansen J.A., and Mikos A.G. Biocompatibility and degradation of poly(DL-lactic-co-glycolic acid)/calcium phosphate cement composites. Journal of Biomedical Materials Research A 74, 533, 2005.
26. Habraken W.J., Wolke J.G., Mikos A.G., and Jansen J.A. Injectable PLGA microsphere/calcium phosphate cements: physical properties and degradation characteristics. Journal of Biomaterials Science, Polymer Edition 17, 1057, 2006.
27. Veldhuis G., Gironès M., and Bingham D. Monodisperse Microsphere for Parenteral Drug Delivery. Drug Delivery Technology 9, xx, 2009.
28. Bodde E.W., Cammaert C.T., Wolke J.G., Spauwen P.H., and Jansen J.A. Investigation as to the osteoinductivity of macroporous calcium phosphate cement in goats. Journal of Biomedical Materials Research Part B: Applied Biomaterials 83, 161, 2007.
29. Habraken W.J., Wolke J.G., Mikos A.G., and Jansen J.A. PLGA microsphere/calcium phosphate cement composites for tissue engineering: in vitro release and degradation characteristics. Journal of Biomaterials Science, Polymer Edition 19, 1171, 2008.
30. Tracy M.A., Ward K.L., Firouzabadian L., Wang Y., Dong N., Qian R., and Zhang Y. Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro. Biomaterials 20, 1057, 1999.
31. Zolnik B.S. and Burgess D.J. Effect of acidic pH on PLGA microsphere degradation and release. Journal of Controlled Release 122, 338, 2007.
32. Browaeys H., Bouvry P., and de Bruyn H. A literature review on biomaterials in sinus augmentation procedures. Clinical Implant Dentistry and Related Research 9, 166, 2007.
33. Gosain A.K., McCarthy J.G., Staffenberg D., Glat P.M., and Simmons D.J. The histomorphologic changes in vascularized bone transfer and their interrelationship with the recipient sites: a 1-year study. Plastic and Reconstructive Surgery 97, 1001, 1996.
34. Huse R.O., Quinten R.P., Wolke J.G., and Jansen J.A. The use of porous calcium phosphate scaffolds with transforming growth factor beta 1 as an onlay bone graft substitute. Clinical Oral Implants Research 15, 741, 2004.
35. Tamimi F.M., Torres J., Tresguerres I., Clemente C., Lopez-Cabarcos E., and Blanco L.J. Bone augmentation in rabbit calvariae: comparative study between Bio-Oss and a novel beta-TCP/DCPD granulate. Journal of Clinical Periodontology 33, 922, 2006.
36. Marino F.T., Torres J., Tresguerres I., Jerez L.B., and Cabarcos E.L. Vertical bone augmentation with granulated brushite cement set in glycolic acid. Journal of Biomedical Materials Research A 81, 93, 2007.
37. Alkhraisat M.H., Rueda C., Jerez L.B., Tamimi M.F., Torres J., Gbureck U., and Lopez C.E. Effect of silica gel on the cohesion, properties and biological performance of brushite cement. Acta Biomaterialia 6, 257, 2010.
38. Habraken W.J., Liao H.B., Zhang Z., Wolke J.G., Grijpma D.W., Mikos A.G., Feijen J., and Jansen J.A. In vivo degradation of calcium phosphate cement incorporated into biodegradable microspheres. Acta Biomaterialia 6, 2200, 2010.
39. Bohner M. and Baumgart F. Theoretical model to determine the effects of geometrical factors on the resorption of calcium phosphate bone substitutes. Biomaterials 25, 3569, 2004.

40. von Doernberg M.C., von Rechenberg B., Bohner M., Grunenfelder S., van Lenthe G.H., Muller R., Gasser B., Mathys R., Baroud G., and Auer J. In vivo behavior of calcium phosphate scaffolds with four different pore sizes. Biomaterials 27, 5186, 2006.
41. Lu J.X., Flautre B., Anselme K., Hardouin P., Gallur A., Descamps M., and Thierry B. Role of interconnections in porous bioceramics on bone recolonization in vitro and in vivo. Journal of Materials Science: Materials in Medicine 10, 111, 1999.

# CHAPTER 06

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# CHAPTER 06

## Maxillary sinus floor augmentation with injectable calcium phosphate cements: A pre-clinical study in sheep

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# INTRODUCTION

Throughout the history of dentistry, dentists and oral-maxillofacial surgeons have thought of ways to replace missing teeth. Loss of teeth can be caused by trauma or disease and is known to be related to age, ethnicity, health condition, poverty and educational status.[1] Despite increased oral healthcare and prevention over the last decades, full edentulism is still observed for around 4% of people in the age of 20 to 64 in the United States of America.[1]

Treatment options for tooth loss have evolved from traditional fixed or removable (partial) dentures to implant dentistry in the last few decades. Treatment with dental implants has the ability to maintain the alveolar ridge over time, which is known to resorb in height and width after extraction of teeth.[2-4] Furthermore, in the maxillary (pre)molar region the presence of the maxillary sinus may even further compromise the possibility for primary implant placement.[5] Therefore, it is a major challenge in implant dentistry to provide treatment modalities that regain or maintain original alveolar ridge dimensions that enable implant placement, especially in the maxillary (pre)molar region.

To regenerate bone of sufficient quality and quantity for the installation of dental implants in the region of the maxillary (pre)molars, maxillary sinus floor elevation procedures can be performed.[5] Autologous bone is considered to be the gold standard grafting material in these procedures.[6-8] However, the disadvantages involved with harvesting autologous bone (e.g. an additional surgery site, extra time, and extra morbidity) and the general limited availability, emphasize the need for synthetic bone substitution materials.

Calcium phosphate (CaP) based materials are widely used as synthetic bone substitutes in the field of dentistry, orthopedics and reconstructive surgery.[6;8] From a clinical perspective, CaP based materials in an injectable formulation have several advantages over pre-fabricated CaPs, including ease of handling, application through minimally invasive surgical techniques, and the ability to be fully adapted to the dimensions of the bone defect. Nevertheless, the most important disadvantage of these calcium phosphate cements (CPCs) remains their lack of controlled degradation to allow replacement by newly formed bone.[9]

To increase the degradation of CPCs and hence allow bone regeneration, efforts have focused on various methods to introduce macroporosity within CPCs.[9] An appealing approach is the incorporation of biodegradable polymeric microspheres within CPCs, which after hydrolytic or enzymatic degradation generate pores within the CPC matrix.[10] Polymeric microspheres based on poly(D,L-lactic-*co*-glycolic)acid (PLGA) have gained most interest for this purpose, as PLGA has a long clinical history and such CPC-PLGA composites have demonstrated to be biocompatible in several animal experiments.[11] In a CPC-PLGA composite, PLGA

microspheres degrade in a relatively short time period by hydrolysis resulting in porosity that increases the surface area of the CPC matrix and as such accelerates degradation of the CPC.[12] Control over CPC-PLGA composite degradation can be obtained by varying the type and chemical characteristics of the PLGA used for the preparation of microspheres.[13-15] Low molecular weight PLGA (LMW; <20 kDa) in CPC-PLGA composites has been shown to accelerate composite degradation as well as new bone formation compared to high molecular weight (HMW; >40 kDa) equivalents.[13-15] In addition to variations in molecular weight, recent studies have shown that chemical modification of PLGA end-groups (i.e. acid-terminated or end-capped) affects CPC-PLGA composite degradation to a larger extent.[14]

In an effort to combine the molecular weight and chemical modification modalities and to test it in a large animal study comparable to the human situation, the aim of this study was to evaluate the biological performance of injectable CPC-PLGA composite materials in a maxillary sinus floor elevation model in sheep. In order to maximize the difference in PLGA degradation properties, PLGA microspheres were made of either LMW acid-terminated PLGA (CPC-PLGA$_{L\text{-}AT}$) or HMW end-capped PLGA (CPC-PLGA$_{H\text{-}EC}$). It was hypothesized that composite degradation and bone formation would be enhanced for CPC-PLGA$_{L\text{-}AT}$ compared to CPC-PLGA$_{H\text{-}EC}$ composites.

# MATERIALS AND METHODS

### Materials

CaP-based cement powder consisted of 85% alpha tri-calcium phosphate (CAM Bioceramics BV, Leiden, the Netherlands), 10% di-calcium phosphate anhydrous (Baker, Griesheim, Germany) and 5% precipitated hydroxyapatite (Merck, Darmstadt, Germany). PLGA was obtained from Purac Biomaterials BV (Gorinchem, the Netherlands) in two variations: Purasorb® PDLG 5002A (MW ~17 kDa, acid terminated end-group functionalization) and Purasorb® PDLG 5002 (MW ~44 kDa, end capped end-group functionalization). Both PLGA types had a lactic-to-glycolic ratio of 50:50. A 0.2 µm filter-sterilized aqueous solution of 2% $Na_2HPO_4$ (Merck, Darmstadt, Germany) was used as the liquid phase for CPC preparation.

### Preparation of PLGA microspheres

Microspheres were prepared by an established water-in oil-in water (w/o/w) double emulsion solvent evaporation technique as described previously.[14] Briefly, 1.0 g of PLGA was dissolved in 4 mL of dichloromethane (Merck, Darmstadt, Germany). After dissolution of the polymer, 500 mL of demineralized $H_2O$ was added while emulsified vigorously with an Ultra-Turrax® (IKA, Staufen, Germany)

at 8000 rpm. After 90 s of emulsifying, 6 mL of a 0.3% poly vinyl alcohol (PVA) solution (Acros Organics, Geel, Belgium) was added and emulsifying was continued for another 90 s. Then, this content was transferred to a 1000 mL beaker and another 394 mL of 0.3% PVA solution was added slowly under continuous stirring. This was directly followed by adding 400 mL of a 2% isopropylic alcohol solution (Merck, Darmstadt, Germany). PLGA microspheres were allowed to settle for 1.5 h, after which the solution was decanted and PLGA microspheres were collected by centrifugation at 1500 rpm for 5 min. The microspheres were freeze dried for 24 h and finally stored at -20°C.

**Preparation of CPC-PLGA composites**

All material samples were prepared in 10 mL syringes with closed tip (BD Plastipak™, Becton Dickinson S.A., Madrid, Spain) by mixing PLGA microspheres with CaP cement powder in a 20:80 wt% ratio to a total 5.0 g of CPC-PLGA composite per syringe. All material samples were sterilized by γ-radiation with a minimum dose of 25 kGy (Isotron B.V., Ede, the Netherlands). Before adding the liquid phase, the powder component was mixed for 15 s with a standard amalgam mixer (Silamat®, Vivadent, Schaan, Liechtenstein) to distribute PLGA microspheres homogeneously throughout the CaP powder. Subsequently, 1.95 mL of 2% $Na_2HPO_4$ solution was added to the powder component (liquid/powder ratio: 0.39 mL/g) and immediately mixed for 30 s again just before injection. This procedure was used to generate two different CPC-PLGA composites:

CPC-PLGA$_{L\text{-}AT}$ (containing microspheres of LMW PLGA with acid-terminated end-groups); and

CPC-PLGA$_{H\text{-}EC}$ (containing microspheres of HMW PLGA with end-capped end-groups).

**Material characterisation**

Microsphere size distribution of PLGA microspheres was assesed by image analysis. The microspheres were suspended in water and images were taken with an optical microscope (Leica/Leitz DM RBE Microscope system, Leica Microsystems AG, Wetzlar, Germany). Subsequently, the diameter of microspheres was determined (n>250 for each type of PLGA) with image analysis software (Leica Qwin®, Leica Microsystems AG, Wetzlar, Germany).

Total porosity of CPC-PLGA composites (PLGA-induced macroporosity plus intrinsic CaP microporosity) was assesed as described before.[10] Briefly, CPC-PLGA composites were injected in Teflon molds (Ø 7 mm, height 3 mm) and allowed to set for 24 h (n=5). Mass determinations of pre-set and heat-treated CPC-PLGA composites (furnace at 650°C for 2 h) were used to calculate total porosity according to the equation in Figure 1.

$$\varepsilon_{tot} = \left(1 - \frac{m_{burnt}}{V \times \rho_{HA}}\right) \times 100\%$$

Figure 1: Equation to calculate CPC porosity. $\varepsilon_{tot}$ = total porosity (%), $m_{burnt}$ = average sample mass after burning out the polymer (g), $V$ = theoretical volume of the sample ($cm^3$) and $\rho_{HA}$ = theoretical density of pure hydroxy apatite ($g/cm^3$).

### Animals

A total of eight female Swifter sheep in healthy condition (average weight: 65 kg) were used in this study. The experimental protocol was reviewed and approved by the Experimental Animal Ethical Committee of the Radboud University Nijmegen, the Netherlands (RU-DEC 2008-194). National guidelines for the care and use of laboratory animals were observed.

### Surgical procedure

Preoperatively, a single dose of amoxicilline 10 mg/kg i.v. (Albipen®, Intervet BV, Boxmeer, the Netherlands) was administered as profylaxis to reduce infection risk. Surgery was performed under general inhalation anesthesia, initiated by intravenous administration of pentobarbital (AUV Wholesale, Cuijk, the Netherlands). Thereafter, the animals were intubated and connected to an inhalation ventilator with a constant volume mixture of nitrous oxide, isoflurane and oxygen (Rhodia Organique Fine Limited, Avonmouth, Bristol, UK). Each animal was ventrally immobilized and the skin overlying the lateral wall of the maxillary sinus was shaved, washed and disinfected with povidone-iodine. Each sheep underwent a bilateral sinus floor elevation procedure under sterile conditions by means of an extra-oral approach. A split mouth design was used in which each animal received both material samples, alternately installed in the left or right maxillary sinus. The facial bony sinus wall was exposed over a 4 cm long paramedial sagittal skin incision, thereby avoiding to cut the facial artery. The masseter muscle was partially detached and a bony window with a diameter of ~10 mm was created in the lateral maxillary sinus wall under constant saline cooling with a dental burr (Elcomed® 100, W&H Dentalwerk Burmoos GmbH, Bürmoos, Austria). The resultant bone plate was carefully removed from the Schneiderian membrane. The Schneiderian membrane was elevated from the buccal, caudal and medial bony wall and displaced cranially with bent, blunt dissectors. Care was taken during this procedure to avoid tearing or damaging the Schneiderian membrane. A gauze was inserted in the maxillary sinus cavity to optimally dry the cavity and to keep the Schneiderian membrane in its elevated position. CPC-PLGA was injected into the maxillary sinus immediately after removal of the gauze. After setting of the CPC-PLGA composite, the

removed bony window was placed back into position. Soft tissues were closed in separate layers using resorbable sutures (Vicryl™ 3-0, Ethicon, Inc., Somerset County, NJ, USA). Directly after the surgical procedure, a conventional radiograph was taken of the grafted sinuses to verify augmentation location. Flunixide 2 mg/kg/24h i.m., 3 doses, (Finadyne®, AUV Wholesale, Cuijk, the Netherlands) and amoxicilline 15 mg/kg/48h, 2 doses, (Albipen-LA®, Intervet BV, Boxmeer, the Netherlands) were administered to reduce post-operative pain and infection risk, respectively. Directly following sinus augmentation surgery and after 6 weeks of healing time, a radiograph was obtained of the maxillary sinus area to verify graft localization and surgery.

**Sequential fluorochrome labeling**

To visualize the dynamics of bone growth, fluorochrome markers were administered subcutaneously to seven sheep (to analyze auto-fluorescence after histological processing, the eighth sheep was used as a control). Sequential fluorochrome labels were administered at 1 week (oxytetracycline, blue; 25 mg/kg), 3 weeks (alizarin complexon, red; 25 mg/kg), 6 weeks (calcein, green; 25 mg/kg) and 9 weeks (oxytetracycline, blue; 25 mg/kg) post-surgery.

**Sample retrieval and histological processing**

All eight animals were sacrificed using an overdose of pentobarbital after 12 weeks. A total of 16 maxillary sinuses could be retrieved and were excised. Subsequently, the retrieved specimens were fixed in a 4% phosphate-buffered formalin solution for 72 h. Specimens were dehydrated in a graded series of ethanol (70-100%) and embedded in poly(methylmethacrylate). After polymerization, ~10 µm thick bucco-palatal sections (5 sections of each specimen) were prepared using a sawing microtome technique with a diamond blade (SP 1600, Leica Microsystems AG, Wetzlar, Germany). Three histological sections per specimen were stained with methylene blue and basic fuchsin for histological and histomorphometrical analysis. Two sections per specimen were left unstained for fluorochrome labeling analysis.

**Histological, histomorphometrical and fluorochrome labeling analysis**

Digital images of all histological sections were made using a light microscope (Axio Imager.Z1, Carl Zeiss AG Light Microscopy, Göttingen, Germany). Histological analysis consisted of a concise morphological and histological description of all sections. Computer-based quantitative image analysis techniques (Leica QWin Pro®, Leica Microsystems AG, Wetzlar, Germany) were used for histomorphometrical analysis of all digital images of the stained sections.

Figure 2 shows the different areas that were identified in the sections. The region of interest (ROI) was defined as the total grafted area (without the original maxillary sinus wall). The ROI was manually set using Leica QWin Pro® software and consisted of both areas C (CPC) and NB (newly formed bone, due to CPC-PLGA composite degradation and subsequent bone-ingrowth) as shown in Figure 2. Newly formed bone was discriminated from the original maxillary sinus bone wall (represented by SW in Figure 2) by difference in staining intensity and cellular orientation. The following parameters were evaluated:

-Bone-CPC contact (expressed as a percentage of the total cement perimeter)
-Bone area (percentage of bone within the ROI)
-Cement area (percentage of CPC within the ROI)
-Degradation distance (the maximal distance in µm between the original maxillary sinus wall and CPC surface, perpendicular to the newly formed bone-original maxillary sinus wall interface). The degradation distance was measured at three different visually maximal degradation locations per histological section, the largest distance of which was thereafter considered to be the maximal degradation distance.

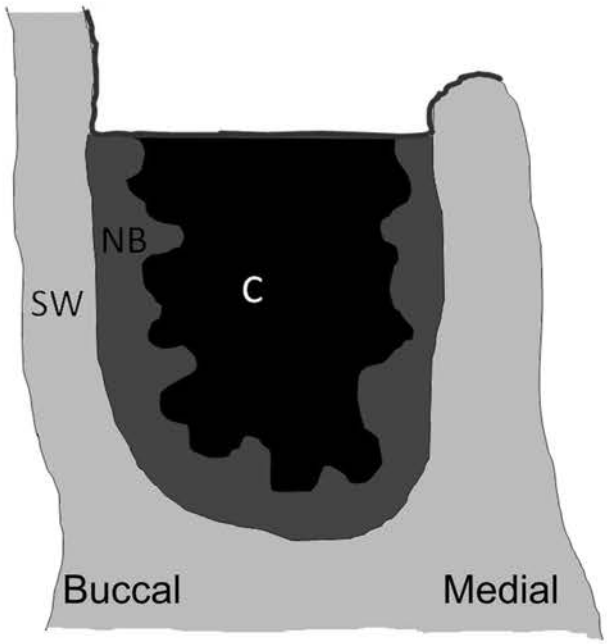


Figure 2: Schematic illustration of the grafted maxillary sinus after 12 weeks of implantation time. SW = pre-existent sinus wall; NB = newly formed bone; C = cement. The Schneiderian membrane is indicated by the red line on top of the grafted area. The region of interest (ROI) was set as both areas NB and C.

Analysis of sequential fluorochrome labeling was performed using a fluorescence microscope (Axio Imager.Z1, Carl Zeiss AG Light Microscopy, Göttingen, Germany). Excitation wavelengths for each of the fluorochrome labels were 365-490 nm / 520-570 nm (blue), 530-580 nm / 600-645 nm (red) and 436-495 nm / 517-540 nm (green). Four images of the same area in each unstained section were taken: one for each fluorochrome marker (oxytetracycline blue, alizarin complexon red and calcein green), the fluorescence images were thereafter merged with one regular image using transmission light microscopy to assess the dynamics of bone formation over time.

### Statistical Analysis

Statistical analysis of data was performed using Instat® 2000 software (version 3.05, GraphPad Software Inc., San Diego, USA). Histomorphometrical data were collected based on three sections per sample and combined to one value per sample. All values were expressed as mean ± standard deviation (SD) and median and analyzed by means of a Student's t-test. To test if data were normally distributed, the method of Kolmogorov and Smirnov was used. The level of significance was set at $p<0.05$.

## RESULTS

### Material characterization

Analysis of the microsphere size distribution revealed similar sizes for microspheres generated from $PLGA_{L\text{-}AT}$ (37 µm ± 11 µm) or $PLGA_{H\text{-}EC}$ (41 µm ± 10 µm). The total porosity of CPC-$PLGA_{L\text{-}AT}$ (67.0% ± 0.41%) was statistically significantly lower ($p$=0.0018) compared to that of CPC-$PLGA_{H\text{-}EC}$ (69.3% ± 1.04%).

### General observations of the animals

The surgical procedure was uneventful for all animals, none of the Schneiderian membranes were damaged or torn and injection of CPCs went uneventful. Directly following sinus augmentation and after 6 weeks of healing time, no dislocations or adverse effects were observed on radiographs (data not shown). Moreover, the post-operative period elapsed without any clinical signs of discomfort. All animals remained in good health during the entire implantation period. During sample retrieval after the 12 week implantation period, no macroscopically visual adverse tissue reaction or clinical signs of inflammation could be detected and all 16 maxillary sinuses could be retrieved.

### Histological analysis

All injected CPC-PLGA composite grafts remained at the original defect site during the entire implantation period. Light microscopy showed newly formed bone from the original bone surface and Schneiderian membrane in both CPC-PLGA composite groups (Figure 3). Occasional cracks and voids in the grafts were filled with newly formed bone tissue. A direct contact between CPC-matrix and newly formed bone was observed. The Schneiderian membrane was in close contact with the CPC-PLGA composites and/or newly formed bone tissue (Figure 3a and 4a). At a higher magnification, newly formed bone appeared as vital bone tissue, containing osteoclasts, osteoid, and osteocytes inside bone lacunae (Figure 4c). Newly formed bone could be identified in close vicinity to the cement surface. At a greater distance from the cement surface, bone-marrow like tissue was observed, including osteoid and osteoblasts (Figure 4b).

CPC-PLGA$_{L\text{-}AT}$ showed more apparent material degradation and an irregular cement perimeter compared to samples containing CPC-PLGA$_{H\text{-}EC}$ (Figure 3). Degradation of both types of CPC-PLGA composites was characterized by the occurrence of occasional free cement particles in bone marrow cavities and frequent presence of multi-nucleated cells at the cement surface (Figure 4c).

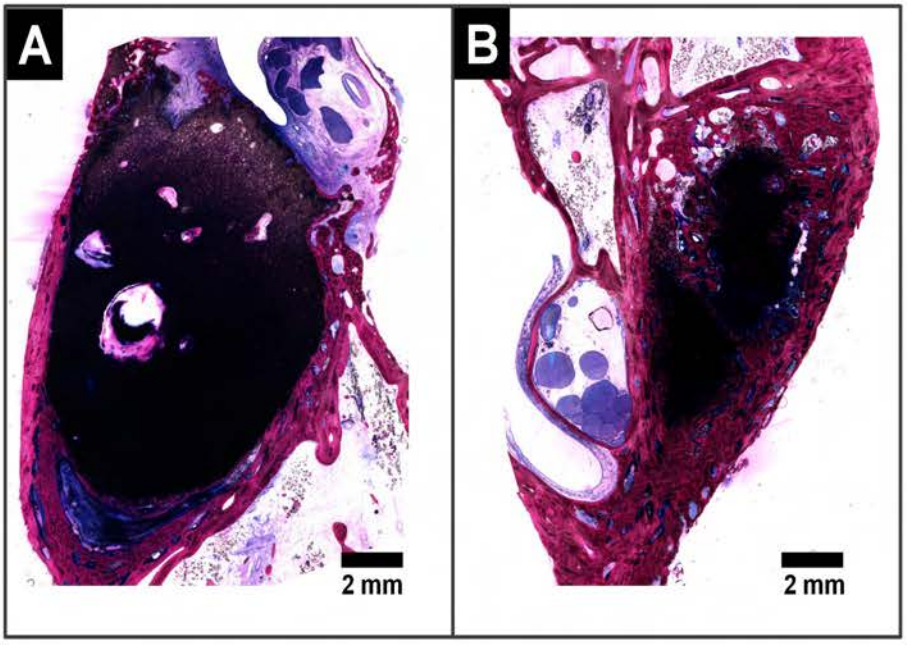


Figure 3: Histological overview of the sinus area (hematoxylin-eosin staining of coronal histological sections after 12 weeks). CPC-PLGA composite (black) embedded in newly formed bone within the maxillary sinus of sheep. (a) CPC-PLGA$_{H\text{-}EC}$ (b) CPC-PLGA$_{L\text{-}AT}$.

### Histomorphometrical analysis

The method of Kolmogorov and Smirnov showed that all presented data of the histomorphometrical analysis were normally distributed. CPC-PLGA$_{L\text{-}AT}$ showed a significantly higher ($p$=0.0009) amount of newly formed bone within the ROI compared to CPC-PLGA$_{H\text{-}EC}$ (26.4% ± 10.5% [median: 27.8%] versus 8.6% ± 3.9% [median: 8.1%], respectively; Figure 5a). Additionally, CPC-PLGA$_{L\text{-}AT}$ showed significantly less ($p$=0.0192) remaining CPC in the ROI than CPC-PLGA$_{H\text{-}EC}$ (61.2% ± 17.7% [median: 61.2%] versus 81.9% ± 10.9% [median: 82.3%], respectively; Figure 5a). Moreover, a significantly increased ($p$=0.0107) degradation distance was found for CPC-PLGA$_{L\text{-}AT}$ (Figure 5b) with a degradation distance from the original maxillary sinus wall of 1949 µm ± 1295 µm (median: 1706 µm) compared to 459 µm ± 267 µm (median: 389 µm) for CPC-PLGA$_{H\text{-}EC}$. Similar bone-cement contact values ($p$=0.7527) were determined for CPC-PLGA$_{L\text{-}AT}$ (61.3% ± 21.0% [median: 53.6%]) and CPC-PLGA$_{H\text{-}EC}$ (64.9% ± 21.7% [median: 61.1%]).

### Fluorochrome labeling analysis

Analysis of the fluorochrome labeling showed a regular distribution of color bands following the order of administration. Bone formation occurred from the original sinus wall and Schneiderian membrane in direction of the degrading CPC-PLGA composite (Figure 6). All fluorescent markers could be identified, although alizarin complexon showed a relative reduced intensity.

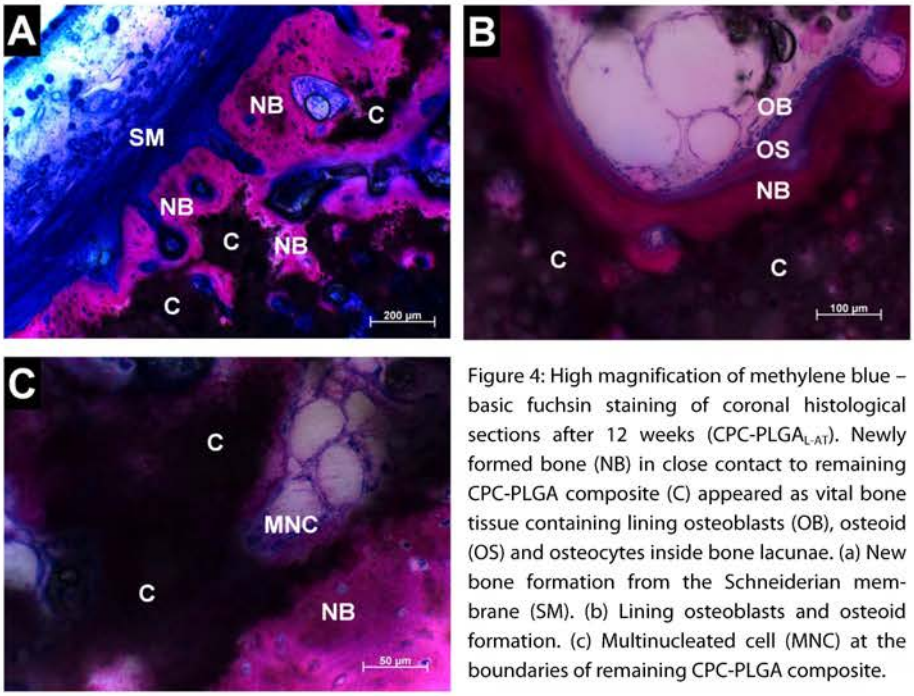


Figure 4: High magnification of methylene blue – basic fuchsin staining of coronal histological sections after 12 weeks (CPC-PLGA$_{L\text{-}AT}$). Newly formed bone (NB) in close contact to remaining CPC-PLGA composite (C) appeared as vital bone tissue containing lining osteoblasts (OB), osteoid (OS) and osteocytes inside bone lacunae. (a) New bone formation from the Schneiderian membrane (SM). (b) Lining osteoblasts and osteoid formation. (c) Multinucleated cell (MNC) at the boundaries of remaining CPC-PLGA composite.

# DISCUSSION

The aim of this pre-clinical study was to evaluate the biological performance of two types of injectable CPC-PLGA composite materials with either rapidly or slowly degrading PLGA microspheres in a maxillary sinus floor elevation model in sheep. Since this study focused solely on evaluating these materials for the first time in a large animal model comparable to a human application, no dental implants were placed. Biocompatibility of both materials was evidenced by the absence of adverse tissue responses and signs of inflammation. Instead, both types of CPC-PLGA showed direct contact to bone without intervening soft tissue layers and degraded CPC-PLGA was gradually replaced by newly-formed bone. Interestingly, CPC-PLGA with rapidly degrading PLGA microspheres showed significantly faster CPC degradation and more bone formation compared to CPC-PLGA with slowly degrading PLGA microspheres, demonstrating the ability to control CPC-PLGA degradation by changing the properties of PLGA microspheres.

A large variety of CPC materials for maxillary sinus augmentation have been described in literature.[6;8] To allow application in a clinical situation, these CPCs need to fulfill a number of criteria amongst which the most important are biocompatibility, biodegradability and easy handling.[9] In previous studies using CPC-PLGA composites in either a pre-set or injectable form in small animal models, excellent biocompatibility has been demonstrated while the material degraded and bone tissue was formed.[13-16] The results of the present study, using a large animal model, corroborate those findings regarding handling, biocompatibility and bone formation.

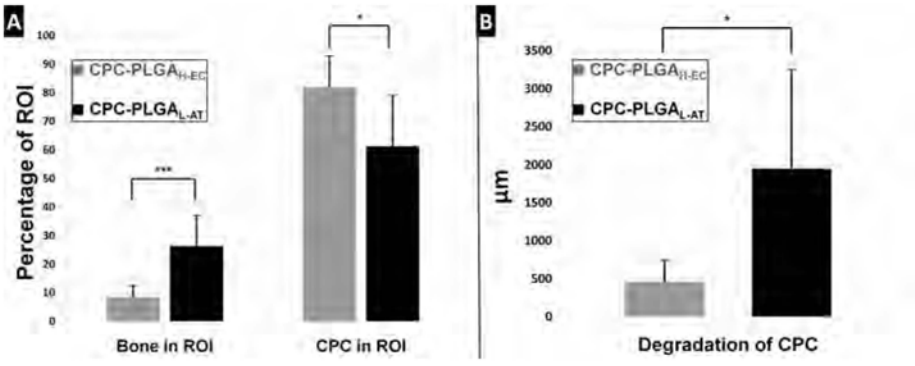


Figure 5: Histomorphometry. (a) Percentage (mean ± SD) of bone and remaining CPC-PLGA composite in the ROI after 12 weeks of implantation time (b) Degradation distance (mean ± SD) of CPC after 12 weeks of implantation time, measured as the maximal distance in µm between the pre-existent maxillary sinus wall and cement, perpendicular to the newly formed bone – pre-existent maxillary sinus wall interface. (* indicates significant difference; $p<0.05$) (*** indicates significant difference; $p<0.001$).

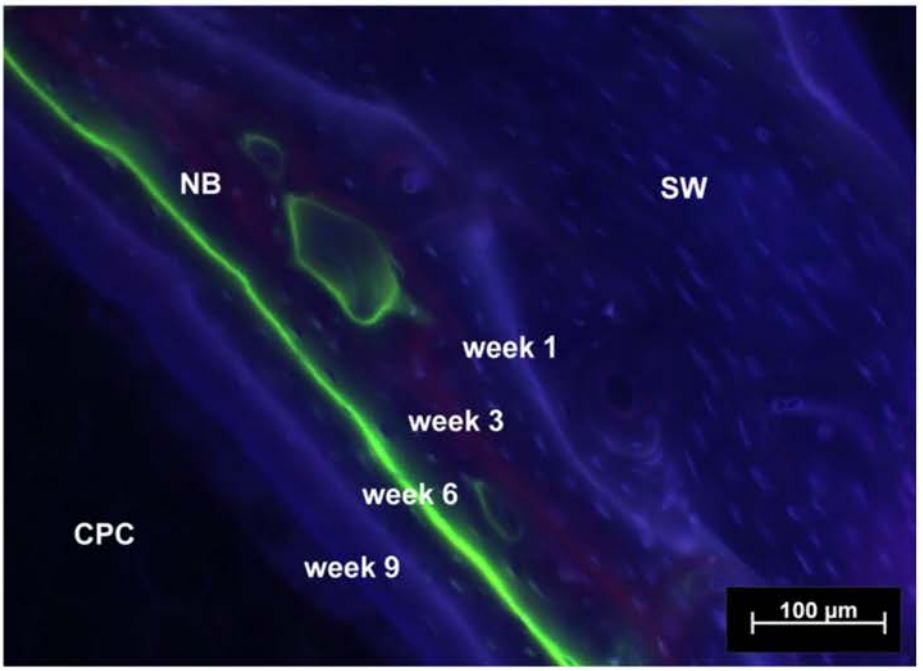


Figure 6: Polychrome sequential fluorochrome labeling. Sequential fluorochrome labels were administered at 1 week (oxytetracycline, blue), 3 weeks (alizarin complexon, red), 6 weeks (calcein, green) and at 9 weeks (oxytetracycline, blue). SW = pre-existent sinus wall; NB = newly formed bone; C = cement.

Degradation of PLGA microspheres is critical for the degradation of CPC-PLGA composites.[14] As control over CPC-PLGA degradation has apparent clinical significance, exploiting variations in PLGA properties has been considered in terms of molecular weight.[13-15] In view of that, Bodde et al. indeed showed that relatively LMW PLGA microspheres evoke an accelerated CPC-PLGA degradation in combination with increased bone formation compared to relatively HMW PLGA equivalents.[13] More recently, the type of PLGA polymer end-group appeared to be a more powerful parameter regarding PLGA degradation and subsequent CaP scaffold degradation.[14] *In vitro* degradation assays by Félix Lanao et al. revealed that acid-terminated PLGA degrades much more rapidly compared to end-capped PLGA.[14] The present study maximized the difference in these PLGA degradation properties by incorporating either microspheres made of LMW and acid-terminated PLGA or HMW and end-capped PLGA into CPC. It was confirmed in this study that rapidly degrading CPC-PLGA allows an increased amount of bone formation compared to the slow degrading equivalent *in vivo*. Solidity and close adaptation to the bony maxillary sinus floor of the CPC-PLGA graft during surgery was confirmed by X-ray images directly post-operative. However, both CPC-PLGA composites showed an irregular

surface at the end of the implantation period with bone formation into the CPC matrix, indicating substantial CPC matrix degradation. The statistically significant difference in porosity between rapidly and slowly degrading CPC-PLGA composites in this study (i.e. 67% vs. 69%) is not likely to be of clinical significance. Van de Watering et al. demonstrated that a higher volumetric PLGA amount in CPC has significant though minimal effects on CPC degradation, and showed similar bone formation for CPC-PLGA composites with a porosity from 64 to 72%.[15] Furthermore, analysis of fluorochrome markers revealed that bone ingrowth occurred in the same direction as CPC degradation took place, i.e. from the original sinus wall towards the center of the grafted area.

The sheep model used in the present study represents analogy with human maxillary sinus floor elevation procedures. Both the required graft volume and shape of the sheep maxillary sinus is comparable to the human situation.[17] However, two major differences exist regarding the maxillary sinus floor elevation procedure in humans versus sheep: the limitation to an extra-oral approach in sheep due to the limited mouth opening and the relatively thick Schneiderian membrane in sheep.[18-20] The latter, however, can be seen as an advantage, since lifting the Schneiderian membrane is less complicated and reduces the risk of damaging or tearing the membrane. In addition, sheep models are a valuable model for human bone turnover and remodeling activity.[21] In support of this theory, Pearce et al. reported that sheep and humans have a similar pattern of bone-ingrowth into porous implants over time.[21] Consequently, the performance of both types of CPC-PLGA composites in the present study is likely to be predictable for the performance in maxillary sinus floor augmentation procedures in humans.

The ultimate goal for the development of a bone substitute material to be used in a clinical setting, is to obtain a material that degrades over time with the same speed as new bone is formed. This process results in a constantly solid grafted area which is replaced from material to natural bone over time. In human cases, the desired pace of bone graft degradation and subsequent replacement by newly formed bone is dependent on the bone forming capacity and moment of implant placement. When commercially available synthetic bone grafts are used in maxillary sinus floor augmentation procedures, usually a broad graft healing period of 4 to 9 months is considered to be required before implants can be installed.[6;8] Therefore, in the present study only one time point was chosen at 12 weeks to assess bone formation after a clinical acceptable healing period. Regarding healing time, the histological results of the present study showed that both types of CPC-PLGA composites are competitive to commercially available synthetic materials,[8] although rapidly degrading CPC-PLGA is the preferred material because already after 12 weeks it showed significantly

enhanced material degradation and a significantly higher amount of bone ingrowth compared to the slow degrading equivalent. Nevertheless, the actual amount of bone formation is still at a clinically insignificant level. Further, the brittleness of CPC-PLGA composite material impedes the (simultaneous) installation of dental implants without jeopardizing the structural integrity of the graft material and implant stability. In previous small animal studies, CPC-PLGA composites demonstrated to be eventually completely degraded and replaced by bone after an implantation period of 12 weeks in rats.[13] However in the present animal model this would require a clinically unacceptable healing period. Therefore, the material characteristics of CPC-PLGA composites need to be optimized further, since the present composition seems to degrade too slow and therefore hinder new bone formation.

Further enhancement of bone growth and speed has been described by the inclusion of several biological active factors to bone graft substitute materials in animal models.[22] In view of this, PLGA microspheres within CPC have the advantageous possibility to function as a drug-delivery vehicle. Several reports have demonstrated the beneficial influence of rhBMP-2 on new bone formation in CPC-PLGA composites.[23-25] Furthermore, a significant increase of bone material contact was observed for injectable CPC-PLGA composites enriched with TGF-β1.[16] However, both recombinant growth factors represent expensive treatment modalities. In addition, this financial aspect becomes even more evident with increasing graft dimensions compared to the use of bare CPC-PLGA composites. Therefore, future research should aim at optimization of CPC-PLGA degradation properties in order to fully control material degradation and replacement by bone tissue.

## CONCLUSION

Both CPC-PLGA$_{L\text{-}AT}$ and CPC-PLGA$_{H\text{-}EC}$ demonstrated to be safe materials for sinus floor augmentation procedures in a large animal model, presenting biocompatibility and direct bone contact. In view of material performance, CPC-PLGA$_{L\text{-}AT}$ showed significantly faster degradation and a significantly higher amount of newly formed bone compared to CPC-PLGA$_{H\text{-}EC}$. However, the degradation rate of CPC-PLGA$_{L\text{-}AT}$ needs to be improved to allow faster and more bone formation in a clinically acceptable time period.

# REFERENCES


1. Dye B.A., Tan S., Smith V., Lewis B.G., Barker L.K., Thornton-Evans G., Eke P.I., Beltran-Aguilar E.D., Horowitz A.M., and Li C.H. Trends in oral health status: United States, 1988-1994 and 1999-2004. Vital and Health Statistics 11, 1, 2007.
2. Lindquist L.W., Carlsson G.E., and Jemt T. A prospective 15-year follow-up study of mandibular fixed prostheses supported by osseointegrated implants. Clinical results and marginal bone loss. Clinical Oral Implants Research 7, 329, 1996.
3. Schropp L., Wenzel A., Kostopoulos L., and Karring, T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. International Journal of Periodontics and Restorative Dentistry 23, 313, 2003.
4. Farina R., Pramstraller M., Franceschetti G., Pramstraller C., and Trombelli L. Alveolar ridge dimensions in maxillary posterior sextants: a retrospective comparative study of dentate and edentulous sites using computerized tomography data. Clinical Oral Implants Research 22, 1138, 2011.
5. McAllister B.S. and Haghighat K. Bone augmentation techniques. Journal of Periodontology 78, 377, 2007.
6. Nkenke E. and Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clinical Oral Implants Research 20 Suppl 4, 124, 2009.
7. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. Sinus floor augmentation surgery using autologous bone grafts from various donor sites: a meta-analysis of the total bone volume. Tissue Engineering Part B Reviews 16, 295, 2010.
8. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. A meta-analysis of histomorphometric results and graft healing time of various biomaterials compared to autologous bone used as sinus floor augmentation material in humans. Tissue Engineering Part B Reviews 16, 493, 2010.
9. Bohner M. Design of ceramic-based cements and putties for bone graft substitution. European Cells & Materials Journal 20, 1, 2010.
10. Habraken W.J., Wolke J.G., Mikos A.G., and Jansen J.A. Injectable PLGA microsphere/calcium phosphate cements: physical properties and degradation characteristics. Journal of Biomaterials Science, Polymer Edition 17, 1057, 2006.
11. Shive M.S. and Anderson J.M. Biodegradation and biocompatibility of PLA and PLGA microspheres. Advanced Drug Delivery Reviews 28, 5, 1997.
12. LeGeros R.Z. Biodegradation and bioresorption of calcium phosphate ceramics. Clinical Materials 14, 65, 1993.
13. Bodde E.W., Habraken W.J., Mikos A.G., Spauwen P.H., and Jansen J.A. Effect of polymer molecular weight on the bone biological activity of biodegradable polymer/calcium phosphate cement composites. Tissue Engineering Part A 15, 3183, 2009.
14. Felix Lanao R.P., Leeuwenburgh S.C., Wolke J.G., and Jansen J.A. In vitro degradation rate of apatitic calcium phosphate cement with incorporated PLGA microspheres. Acta Biomaterialia 7, 3459, 2011.
15. van de Watering F.C., van den Beucken J.J., Walboomers X.F., and Jansen J.A. Calcium phosphate/poly(d,l-lactic-co-glycolic acid) composite bone substitute materials: evaluation of temporal degradation and bone ingrowth in a rat critical-sized cranial defect. Clinical Oral Implants Research 23, 151, 2012.
16. Plachokova A., Link D., van den Dolder J., van den Beucken, J., and Jansen J. Bone regenerative properties of injectable PGLA-CaP composite with TGF-beta1 in a rat augmentation model. Journal of Tissue Engineering and Regenerative Medicine 1, 457, 2007.
17. Estaca E., Cabezas J., Uson J., Sanchez-Margallo F., Morell, E., and Latorre R. Maxillary sinus-floor elevation: an animal model. Clinical Oral Implants Research 19, 1044, 2008.
18. Haas R., Donath K., Fodinger M., and Watzek G. Bovine hydroxyapatite for maxillary sinus grafting: comparative histomorphometric findings in sheep. Clinical Oral Implants Research 9, 107,

1998.

19. Jakse N., Tangl S., Gilli R., Berghold A., Lorenzoni M., Eskici A., Haas R., and Pertl C. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clinical Oral Implants Research 14, 578, 2003.
20. Brumund K.T., Graham S.M., Beck K.C., Hoffman E.A., and McLennan G. The effect of maxillary sinus antrostomy size on xenon ventilation in the sheep model. Otolaryngology - Head & Neck Surgery 131, 528, 2004.
21. Pearce A.I., Richards R.G., Milz S., Schneider E., and Pearce S.G. Animal models for implant biomaterial research in bone: a review. European Cells & Materials Journal 13, 1, 2007.
22. Browaeys H., Bouvry P., and De Bruyn H. A literature review on biomaterials in sinus augmentation procedures. Clinical Implant Dentistry and Related Research 9, 166, 2007.
23. Kroese-Deutman H.C., Ruhe P.Q., Spauwen P.H., and Jansen, J.A. Bone inductive properties of rhBMP-2 loaded porous calcium phosphate cement implants inserted at an ectopic site in rabbits. Biomaterials 26, 1131, 2005.
24. Ruhe P.Q., Boerman O.C., Russel F.G., Spauwen P.H., Mikos A.G., and Jansen J.A. Controlled release of rhBMP-2 loaded poly(dl-lactic-co-glycolic acid)/calcium phosphate cement composites in vivo. Journal of Controlled Release 106, 162, 2005.
25. Bodde E.W., Boerman O.C., Russel F.G., Mikos A.G., Spauwen P.H., and Jansen J.A. The kinetic and biological activity of different loaded rhBMP-2 calcium phosphate cement implants in rats. Journal of Biomedical Materials Research Part A 87, 780, 2008.

# CHAPTER 07

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# CHAPTER 07

## Maxillary Sinus Augmentation with Micro Structured Tricalcium Phosphate Ceramic in Sheep

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# INTRODUCTION

Placement of dental implants requires the presence of adequate alveolar bone quantity and quality. In case the amount of alveolar bone or its quality are insufficient, additional surgical techniques are needed to achieve primary implant stability.[1] For extensive alveolar defects, onlay or inlay grafting procedures have been advised.[1-5] To allow implant placement in the posterior part of the maxilla, sinus floor augmentation surgery has become a routine procedure[6-9] that results in an implant survival rate of over 90 percent for 3 to 5 years.[10;11]

Autologous bone grafts are considered the gold standard in sinus floor augmentation.[12;13] However, harvesting an autologous bone graft, especially from extra-oral sources, is associated with several disadvantages. Especially, reservations must be made regarding the prolonged operating time and donor site morbidity,[14-17] which may include hypersensitivity,[18] pelvic instability, infection,[19;20] and paraesthesia.[21] Consequently, various allogenic, xenogenic and synthetic graft materials or combinations thereof have been used as an alternative to autologous bone grafts with variable clinical results.[11;13;22]

Meta-analysis of augmented maxillary sinuses demonstrated comparable newly formed bone volumes for different types of biocompatible, osteoconductive bone substitutes, mostly calcium phosphate (CaP) ceramics.[13] In an advanced set-up, such synthetic CaP-based bone substitutes have been combined with autologous bone, growth factors or even as a fully tissue engineered cellular construct to establish osteoinductive capacity.[12;13] These composites combine the advantages of each element alone, i.e. osteoconductive properties from the synthetic material and osteoinductive capacity from biological components. Nevertheless, the disadvantage of harvesting bone or the expenses for using growth factors are still present using this approach. Furthermore, the efficacy of cell-based constructs remains unclear as evidenced by conflicting experimental results reported using various cell types of animal or human origin.[23;24]

In view of safety, regulatory and application issues, the ideal synthetic bone substitute should be available off-the-shelf and have intrinsic osteoinductive capacity. Winter and Simpson were the first to report ectopic bone formation induced by a biomaterial.[25] Since then, several authors reported material-induced osteogenesis in soft tissues in different animal models.[26;27] A recent study of Yuan et al. showed an excellent osteoinductive capacity of microstructured tricalcium phosphate (MSTCP) particles after 12 and 52 weeks of intramuscular implantation in dogs and sheep.[28;29] Consequently, it was hypothesized that MSCTP particles represent a suitable bone substitute in maxillary sinus floor augmentation surgery.

In view of this, the present study aimed to evaluate the biological performance of MSTCP particles with osteoinductive capacity[28] in maxillary sinus floor augmentation procedures in sheep.

# MATERIALS AND METHODS

## Material

MSTCP particles were kindly provided by RevisiOs BV (Bilthoven, The Netherlands). Production of the particles was described before.[28] In brief, calcium phosphate powders (with a Ca/P ratio 1.5) were mixed with a $H_2O_2$ solution and naphthalene particles to produce slurries. After foaming, drying and evaporation of the naphthalene, the material was sintered for 8 hrs at 1100°C. X-ray diffraction analysis of MSTCP showed more than 90% βTCP phase and a trace of hydroxyapatite (<10wt%).[28] After milling, ceramic particles with a size of 150 to 500 µm were sieved, cleaned and sterilized using gamma irradiation (Isotron Nederland BV, Ede, the Netherlands).

## Animals

In total, eight female Swifter sheep in healthy condition (average weight: 65 kg) were used in this study. The experimental protocol was reviewed and approved by the Experimental Animal Ethical Committee of the Radboud University Medical Center, The Netherlands (RU DEC 2008-194). National guidelines for the care and use of laboratory animals were observed.

## Surgical procedure

To reduce the perioperative infection risk, prophylactic antibiotics were administered subcutaneously (Albipen® 15%, 3ml/50 kg preoperative and Albipen® LA, 7.5ml/50 kg for 3 days post-operative, Intervet BV, Boxmeer, the Netherlands). General anesthesia was initiated by an intravenous injection of pentobarbital (AUV Wholesale, Cuijk, the Netherlands). Subsequently, the sheep were intubated and connected to an inhalation ventilator with a constant volume of a mixture of nitrous oxide, isoflurane, and oxygen. The animals were immobilized in a ventral position and the operation site was shaved, washed and disinfected with povidone-iodine. Access to the maxillary sinus was obtained by first exposing the facial antral wall over a 4 cm long paramedian sagittal skin incision, taking care to avoid the facial artery. After reflecting the skin flap, a bony window with a diameter around 10 mm was created rostrally with a dental burr (Elcomed® 100, W&H Dentalwerk GmbH, Bürmoos, Austria) under continuous external cooling, followed by careful removal of the resultant bone plate from the Schneiderian membrane. The membrane was then elevated from the buccal and caudal bony wall and displaced cranially with bent blunt dissectors. In total eight maxillary sinuses were unilaterally grafted with 2 mL of the MSTCP particles. The grafted defect was covered by the bone plate. Thereafter, the soft tissues were closed in separate layers. To reduce pain after surgery, all sheep received Finadyne® (AUV Wholesale, Cuijk, the Netherlands) for 3 days postoperatively. Directly after sinus

augmentation surgery and after 6 weeks of healing time a radiograph was obtained of the maxillary sinus area to verify graft localization and surgery.

**Sequential fluorescent labeling**

A polychrome sequential fluorescent labeling method was carried out in seven sheep to visualize the dynamics of bone growth, one sheep was used as control to exclude auto fluorescence of the specimens and implanted MSTCP after histological processing. Fluorescent labels oxytetracycline (blue), alizarin complexon (red), calcein (green) and tetracycline (blue) were administered subcutaneously (25 mg/kg body weight) at 1, 3, 6, and 9 weeks post surgery, respectively.

**Sample retrieval and histological processing**

Animals were sacrificed using a overdose of Pentobarbital after 12 weeks post surgery. The maxilla with surrounding tissue was retrieved. Subsequently, the sinus region was excised and excess tissue was removed. By using a diamond saw, the tissues were sawed into smaller blocks suitable for histological processing. Specimens were fixed in phosphate-buffered formaldehyde solution (pH 7.4), dehydrated in a graded series of ethanol (70–100%) and finally embedded in polymethymetacrylate (PMMA). Multiple histological sections (n≥3; ~20 µm) were prepared in a buccal palatinal direction at consecutive levels through the grafted area using a microtome with diamond blade (Leica Microsystems SP 1600, Nussloch, Germany). Histological sections were stained with methylene blue and basic fuchsin. Two unstained sections (~20 µm) were prepared for fluorescent microscopy of each block. Furthermore, MSTCP granules were embedded in PMMA, after which sections (~20 µm) were prepared of the as-received granules before implantation.

**Radiological evaluation**

After sacrifice, cone beam computed tomograms (CBCT) (i-CAT™ 3-D Imaging System, Imaging Sciences International Inc, Hatfield, PA, USA) were made of the maxillae, during which grafts were localized and the maximum augmentation height was measured at three levels with I-Cat Vision® software (Imaging Sciences International, Inc. Hatfield, PA, USA).

**Histological and histomorphometrical analysis**

The histological evaluation consisted of a morphological description of at least three sections of each grafted area using a light microscope (Leica Microsystems AG, Wetzlar, Germany). Fluorescent labeling was observed with unstained sections using a fluorescent microscope (Leica Microsystems AG, Wetzlar, Germany). Excitation wavelengths for each of the fluorescent labels were as follows: 365-490nm/520-570nm (blue), 530-580nm/600-645nm (red) and 436-

495nm/517-540nm (green). The fluorescence images were merged with one regular image using transmission light microscopy to assess the dynamics of bone formation over time.

In addition, the stained sections were quantitatively scored using computer-based image analysis techniques (Leica Qwin Pro-image analysis system). Three randomly selected standardized areas (1.4 $mm^2$) within the boundaries of the grafted area of at least three histological sections were analyzed of each specimen (Figure 1 & 2b). The area of newly formed bone, residual MSTCP and connective tissue were determined and expressed as a percentage within this region of interest by manual selection based on pixel value detection. Furthermore, the perimeter of the granules in direct contact with bone was measured and expressed as a percentage of the total perimeter for ~500 randomly chosen particles from different histological sections. Additionally, the surface, perimeter, and longest axis of these granules in the histological sections were compared with sections of the granules before implantation.

**Statistical analysis**

Quantitative measurements were expressed as median and mean ± standard deviation (mean ± SD). The differences in particle surface, perimeter and longest axis were analyzed by Mann Whitney test. Statistical analysis was performed using SPSS statistical software package (IBM® SPSS 16.0). Data were considered significant at $p<0.05$.

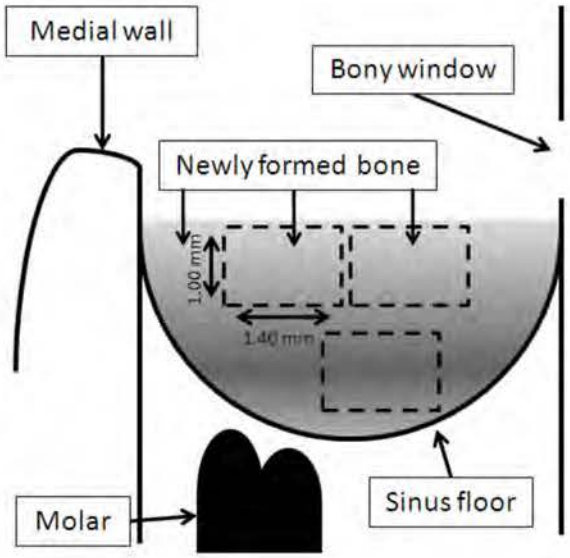


Figure 1: Schematic overview of three randomly selected standardized areas (1.4 $mm^2$) within the boundaries of the grafted area for histomorphometric analysis.

# RESULTS

### General observations of the animals

The surgical procedure was uneventful for all animals. At sacrifice, a total of 8 maxillary sinuses including surrounding tissue could be retrieved. Macroscopically, no signs of infection or adverse tissue reaction were observed.

### Radiological evaluation

Directly after sinus augmentation and after 6 weeks of healing time, an x-ray was obtained of the maxillary sinus area. The location of the surgical site and graft location were examined by comparison. No dislocations or adverse effects were observed (data not shown). A mean augmented height of 6.0±2.2mm was found in CBCT scans after 12 weeks of implantation time.

### Histology

No signs of inflammation or adverse tissue reactions were observed. Bony structures were preserved and the maxillary sinus did not change in shape. Newly formed woven bone was observed bridging the space between the original bone of the buccal wall and sinus floor (Figure 2a). This bone appeared as vital bone tissue containing osteoblasts, osteoid covering the border, and osteocytes inside bone lacunae. Furthermore, bone marrow like tissue was observed in between the bone voids, including blood vessels. The Schneiderian membrane was completely covering the augmented sinus floor (Figure 2a). Remaining MSTCP granules could be easily identified in the newly formed bone by its size, shape, and dark color (Figure 2b). The bone was in close contact with the surface of MSTCP granules without the presence of an intervening fibrous tissue layer. In areas, where the remaining MSTCP particles were in contact with bone marrow, occasionally multinucleated cells were observed at the surface of the particles, suggesting the occurrence of cell-mediated resorption (Figure 2c). Overall, MSTCP particles appeared to be reduced in size compared to their original size.

| N≈500 | Area ($mm^2$) | Perimeter (µm) | Longest axis (µm) | Bone particle contact (%) |
|---|---|---|---|---|
| T = 0 | 0.059 ± 0.047 | 1487 ± 803 | 386 ± 159 | |
| T = 12 weeks | 0.027 ± 0.025 | 851 ± 490 | 258 ± 126 | 82.3 ± 7.5 |
| Mann Whitney | $p<0.001$ | $p<0.001$ | $p<0.001$ | |

Table 1: MSTCP particle degradation. MSTCP particle characteristics before and after 12 weeks implantation time in maxillary sinuses of sheep.

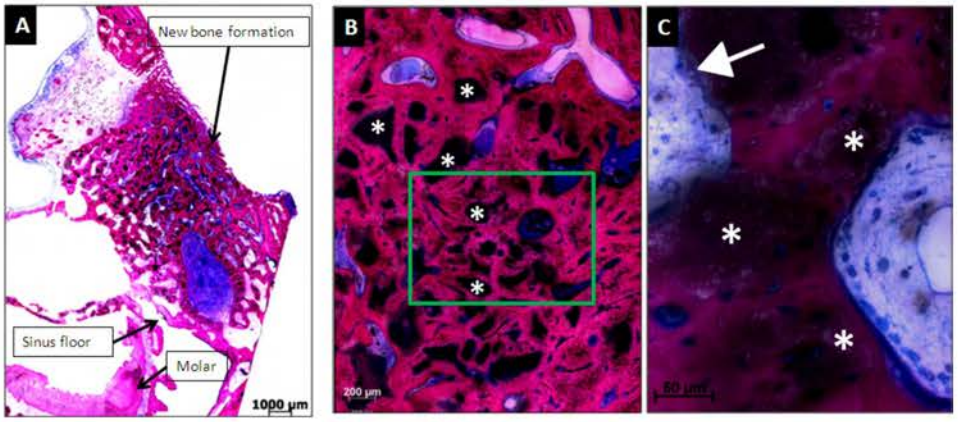


Figure 2: Histology 12 weeks after maxillary sinus augmentation in sheep. Histological overview of the sinus area (A). Higher magnification of the MSTCP particles embedded in bone and direct bone bonding (B); occasionally, multinucleated cells were observed in contact with MSTCP particles (C). Haematoxylin-eosin staining. Green square specify an example of randomly selected area (1.4 mm²) within the boundaries of the grafted area for histomorphometry; * indicates MSTCP particle; arrow indicates multinucleated cell.

## Fluorochrome labeling

Fluorescent labels were systemically administered to allow the visualization of dynamic bone formation at one, three, six and nine weeks. All sequential fluorochrome markers could be identified at a low magnification within the total grafted area in the experimental site (Figure 3). Consecutive fluorochrome markers appearance, laid down in the form of bands, was observed around the MSTCP granules. No clear sequence of fluorescent bands could be observed within the grafted area. In contrast to the labels tetracycline (blue; administered in week 1 and 9) and calcein (green; administered in week 6), the presence of the alizarin complexon label (red; administered in week 3) could not as sharply be identified in the sections.

## Histomorphometry

### *Tissue formation*

Figure 4 demonstrates the results of the histomorphometric analysis of the MSTCP-augmented specimens. The area fraction of the newly formed bone ranged from 25.0% to 65.4%. Randomly selected areas of the histological sections revealed a mean newly formed bone area of 42.9 ± 9.7% (median: 42.3%). Furthermore, a mean area of 33.1±10.9% (median: 23.8%) was occupied by fibrous connective tissue.

***Particle degradation***

Particle surface, perimeter and longest axis were measured before and after implantation to assess particle degradation (Figure 5 & Table 1). A significant ($p<0.001$) decrease of all parameters was measured between the starting material and the MSTCP particles 12 week after implantation (Table 1). The mean residual particle area recorded for MSTCP was 24.0±7.0% (median: 34.0%) after 12 weeks (Figure 4). Furthermore, the percentage of bone in contact with the graft particles was measured to determine the actual bone to particle contact percentage. A mean bone particle contact of 82.3±7.5% (median: 81.7%) was found (Table 1).

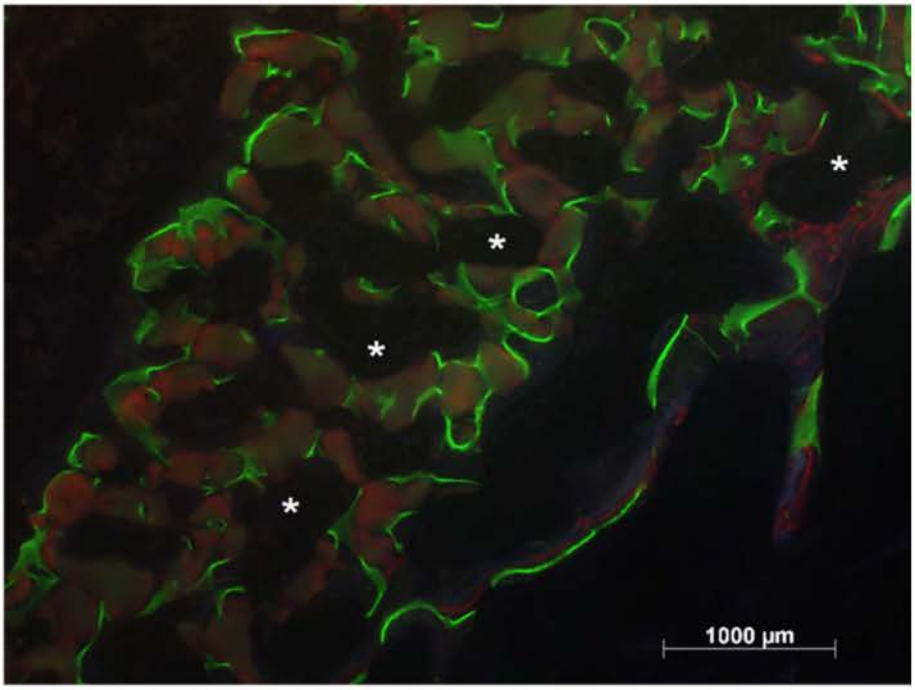


Figure 3: A polychrome sequential fluorescent labeling. Fluorescent labels oxytetracycline (blue), alizarin complexon (red), calcein (green) and tetracycline (blue) after 12 weeks implantation time of the MSTCP group (* indicates MSTCP particle).

# DISCUSSION

This pre-clinical study aimed to evaluate the biological performance of MSTCP particles applied in maxillary sinus augmentation surgery in sheep with an implantation period of 12 weeks. Histology, histomorphometric analysis, sequential fluorescent labeling and maxillofacial CBCT were employed to systematically evaluate new bone formation and bone remodeling in the grafted area. Substantial new bone formation and incorporation of the MSTCP particles in the newly formed bone was observed. These observations indicate a successful performance of MSTCP particles. Additionally, MSTCP particles showed signs of degradation.

Experimental studies on maxillary sinus floor augmentation have been reported using different animal species and various grafting materials. The sheep model is considered one of the suitable larger animal models for maxillary sinus augmentation surgery, due to its similarity in size, bone physiology and structure with the human maxillary sinus.[30] It was, however, not possible to perform the procedure using an intra-oral approach like in humans, due to the limited opening of the mouth of a sheep. Consequently, this model only allows sinus augmentation using an extra-oral approach, as already several authors stated before.[31-34] In contrast to the finding that Schneiderian membrane perforation is a common technical problem in humans,[35] the thickness of the sheep Schneiderian membrane showed enough consistency to perform sinus floor augmentation without tearing the membrane in any case.

Autologous bone grafting is considered the gold standard in sinus floor augmentation surgery.[13] However, a variety of alloplastic bone substitutes, single or in combination with autologous bone, have been used in sinus augmentation surgery in human with various results.[11;13] TCP was already used in the 1970s to heal bone defects.[36] Nkenke et al. analyzed the present literature to determine whether there are advantages of using autologous bone over bone substitutes in sinus floor augmentation procedures with respect to dental implant survival.[11] They concluded that no evidence exists that either supports or refutes the superiority of autologous bone grafts over TCP with regard to dental implant survival.[11] Furthermore, a recent meta-analysis by Klijn et al. demonstrated that histomorphometrically determined bone volumes did not significantly differ from using an autologous bone graft or using TCP in maxillary sinus augmentation.[13] Also, the addition of autologous bone to TCP appeared to have a negligible effect regarding total new bone formation.[13] According to these findings, the MSTCP particles that were used in this preclinical study showed a substantial amount of new bone formation.

The ultimate bone substitute in implant dentistry should eventually be resorbed and replaced by functional newly formed bone. Therefore, the use of resorbable TCP particles for sinus floor elevation has received increasing attention in implant dentistry.[37;38] Resorption of MSTCP particles was demonstrated both histological and histomorphometrically one year after intramuscular implantation in dogs.[28] In the present study, resorption could be confirmed by comparing particle size, perimeter and longest axis before and after implantation in the bony maxillary sinus environment. Yuan et al. demonstrated both chemical dissolution and cell-mediated resorption over time.[28] Considering the occasional presence of multi-nucleated cells in contact with remaining MSTCP particles, the present study corroborates with these findings.

In the past years, several authors described the use of osteoconductive βTCP in human maxillary sinus floor augmentation procedures.[37;39-44] Histomorphometrically obtained bone volumes of 17 to 52% were found in patients after 6 to 12 months of healing time. Besides, the use of TCP has been evaluated in maxillary sinus augmentation in various animal species. In a recent study of Wang et al., TCP was used as grafting material for maxillary sinus augmentation in dogs.[46] A mean newly formed bone area of 34% was found after 24 weeks of healing time. In another study of Jiang et al., TCP was used in sinus augmentation in rabbits, after 8 weeks of healing time a mean newly formed bone area of 16% was found.[47] TCP was also used in a miniature pig study, as described by Gruber,[48]

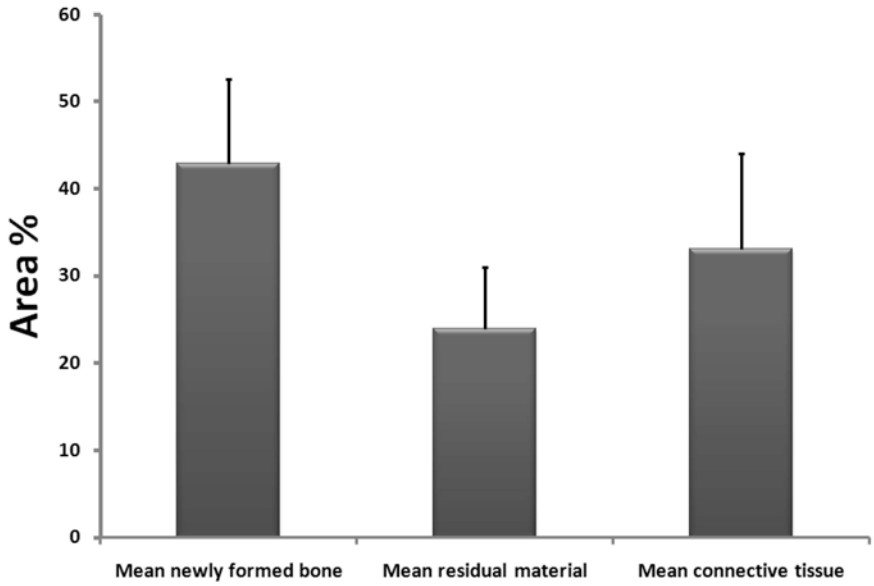


Figure 4: Histomorphometry. Mean percentage of newly formed bone, residual material and connective tissue area after 12 weeks implantation time in maxillary sinuses of sheep.

who installed, in contrast to the current study, a dental implant simultaneously with sinus floor augmentation. A mean bone volume up to 19% was found after 12 weeks.[48] The MSTCP particles evaluated in this study in sheep, resulted in 43% of newly formed bone after 12 weeks after implantation within the grafted area.

Zerbo et al. concluded that due to the absence of osteoinductive properties of the TCP they investigated, the rate of bone formation was delayed in comparison to autologous bone grafting.[42] It would be beneficial for the patient to reduce the interval between maxillary sinus augmentation and implant placement by accelerating the process of integration of the grafted material. Some authors state that the application of osteoinductive substances, such as platelet-rich plasma or growth factors are promising options.[49;50] However, PRP seemed not to be beneficial for new bone formation in sinus augmentation and the use of recombinant growth factors is an expensive option.[51] With the ability to form bone in soft tissue,[28] the osteoinductive MSTCP particles used in this study will be useful to speed up the process of appositional bone growth. Sequential fluorochrome markers were observed within the total grafted area implicating bone growth through the hole specimen already at least 3 weeks after implantation. However, the question remains what healing period is necessary to provide adequate bone formation for successful dental implant placement. Furthermore, MSTCP particles were not evaluated at an ectopic site in this study. Therefore, no conclusions regarding the osteoinductive properties could be drawn. Follow-up studies with prolonged evaluation periods and the application of a combined approach with both sinus floor augmentation and one or two staged implant placement will give an insight in bone to dental implant contact and mechanical stability of the augmented bone.

## CONCLUSION

Based on the histological and histomorphometrical results of this preclinical study, MSTCP particles showed to represent a suitable bone substitute material for maxillary sinus augmentation. The MSTCP demonstrated to provide a scaffold for cell ingrowth and substantial bone formation. Additionally, MSTCP particles showed significant signs of degradation after 12 weeks of implantation in the sheep maxillary sinus.

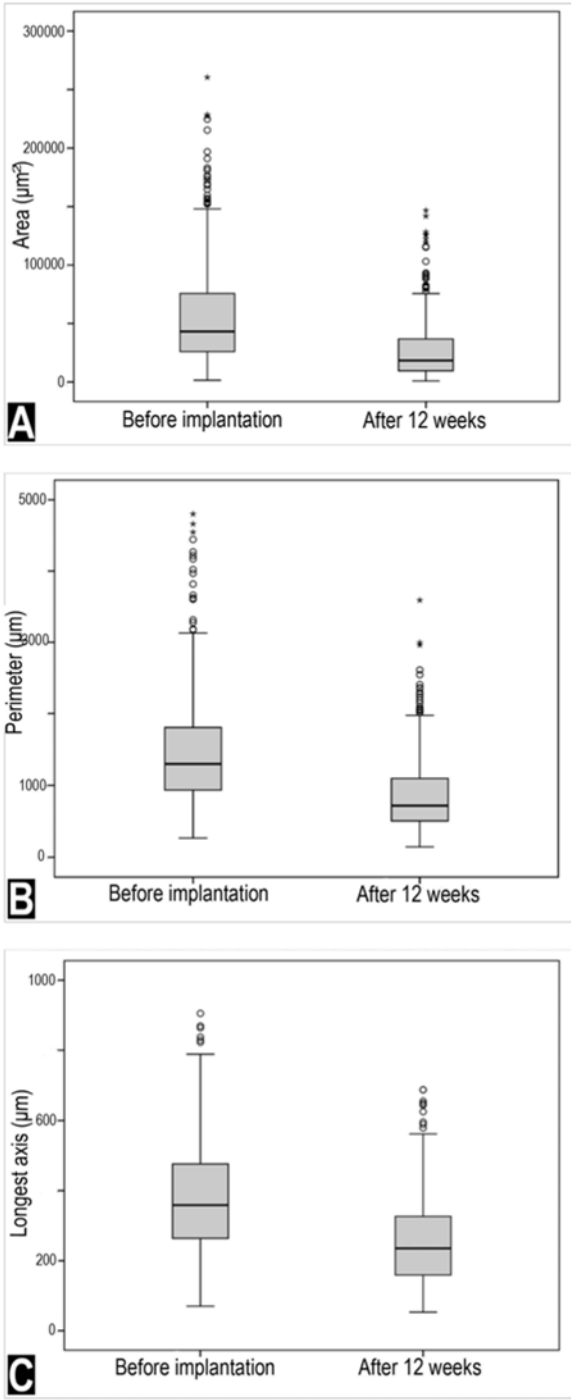


Figure 5: Box plot of MSTCP particle degradation. MSTCP particle characteristics before and after 12 weeks implantation time in maxillary sinuses of sheep. (A) MSTCP particle area (median: 43153 µm²) (B) MSTCP particle perimeter (median: 1300 µm) (C) MSTCP particle longest axis (median: 358 µm).

# REFERENCES


1. Kaufman E. Maxillary sinus elevation surgery: an overview. Journal of Esthetic and Restorative Dentistry 15, 272, 2003.
2. Boyne P.J. and James R.A. Grafting of the maxillary sinus floor with autogenous marrow and bone. Journal of Oral Surgery 38, 613, 1980.
3. Misch C.E. Maxillary sinus augmentation for endosteal implants: organized alternative treatment plans. International Journal of Oral Implantology 4, 49, 1987.
4. Tong D.C., Rioux K., Drangsholt M., and Beirne O.R. A review of survival rates for implants placed in grafted maxillary sinuses using meta-analysis. International Journal of Oral & Maxillofacial Implants 13, 175, 1998.
5. Esposito M., Grusovin M.G., Coulthard P., and Worthington H.V. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. International Journal of Oral & Maxillofacial Implants 21, 696, 2006.
6. Merkx M.A., Maltha J.C., and Stoelinga, P.J. Assessment of the value of anorganic bone additives in sinus floor augmentation: a review of clinical reports. International Journal of Oral and Maxillofacial Surgery 32, 1, 2003.
7. Wallace S.S. and Froum S.J. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Annuals Periodontology 8, 328, 2003.
8. del Fabbro M., Testori T., Francetti L., and Weinstein R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. International Journal of Periodontics & Restorative Dentistry 24, 565, 2004.
9. Emmerich D., Att W., and Stappert C. Sinus floor elevation using osteotomes: a systematic review and meta-analysis. Journal of Periodontology 76, 1237, 2005.
10. Hurzeler M.B., Kirsch A., Ackermann K.L., and Quinones C.R. Reconstruction of the severely resorbed maxilla with dental implants in the augmented maxillary sinus: a 5-year clinical investigation. International Journal of Oral & Maxillofacial Implants 11, 466, 1996.
11. Nkenke E. and Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clinical Oral Implants Research 20 Suppl 4, 124, 2009.
12. Browaeys H., Bouvry P., and de Bruyn H. A literature review on biomaterials in sinus augmentation procedures. Clinical Implant Dentistry and Related Research 9, 166, 2007.
13. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. A meta-analysis of histomorphometric results and graft healing time of various biomaterials compared to autologous bone used as sinus floor augmentation material in humans. Tissue Engineering Part B Reviews 16, 493, 2010.
14. Sindet-Pedersen S. and Enemark H. Reconstruction of alveolar clefts with mandibular or iliac crest bone grafts: a comparative study. Journal of Oral and Maxillofacial Surgery 48, 554, 1990.
15. Cohen M., Figueroa A.A., Haviv Y., Schafer M.E., and Aduss H. Iliac versus cranial bone for secondary grafting of residual alveolar clefts. Plastic and Reconstructive Surgery 87, 423, 1991.
16. Hoppenreijs T.J., Nijdam E.S., and Freihofer H.P. The chin as a donor site in early secondary osteoplasty: a retrospective clinical and radiological evaluation. Journal of Cranio-Maxillofacial Surgery 20, 119, 1992.
17. Eppley B.L. Donor site morbidity of rib graft harvesting in primary alveolar cleft bone grafting. Journal of Craniofacial Surgery 16, 335, 2005.
18. Damien C.J. and Parsons J.R. Bone graft and bone graft substitutes: a review of current technology and applications. Journal of Applied Biomaterials 2, 187, 1991.
19. Canady J.W., Zeitler D.P., Thompson S.A., and Nicholas C.D. Suitability of the iliac crest as a site for harvest of autogenous bone grafts. Cleft Palate Craniofacial Journal 30, 579, 1993.
20. Swan M.C. and Goodacre T.E. Morbidity at the iliac crest donor site following bone grafting of the cleft alveolus. British Journal of Oral and Maxillofacial Surgery 44, 129, 2006.
21. Beirne J.C., Barry H.J., Brady F.A., and Morris V.B. Donor site morbidity of the anterior iliac crest

following cancellous bone harvest. International Journal of Oral and Maxillofacial Surgery 25, 268, 1996.

22. Klijn R.J., Meijer G.J., Bronkhorst E.M., and Jansen J.A. Sinus floor augmentation surgery using autologous bone grafts from various donor sites: a meta-analysis of the total bone volume. Tissue Engineering Part B Reviews 16, 295, 2010.
23. Meijer G.J., de Bruijn J.D., Koole R., and van Blitterswijk C.A. Cell-based bone tissue engineering. PLoS Medicine 4, 260, 2007.
24. Park J.B. Use of cell-based approaches in maxillary sinus augmentation procedures. Journal of Craniofacial Surgery 21, 557, 2010.
25. Winter G.D. and Simpson B.J. Heterotopic bone formed in a synthetic sponge in the skin of young pigs. Nature 223, 88, 1996.
26. Yuan H., de Bruijn J.D., Li Y., Feng J., Yang Z., de Groot K., and Zhang X. Bone formation induced by calcium phosphate ceramics in soft tissue of dogs: a comparative study between porous alpha-TCP and beta-TCP. Journal of Materials Science: Materials in Medicine 12, 7, 2001.
27. Yuan H., Yang Z., de Bruijn J.D., de Groot K., and Zhang X. Material-dependent bone induction by calcium phosphate ceramics: a 2.5-year study in dog. Biomaterials 22, 2617, 2001.
28. Yuan H., de Groot K., van Blitterswijk C.A., and de Bruijn J.D. Preparation of a resorbable osteoinductive tricalcium phosphate ceramic.European Cells & Materials Journal 16, 14, 2008.
29. Yuan H., Fernandes H., Habibovic P., de Boer J., Barradas A.M., de Ruiter A., Walsh W.R., van Blitterswijk C.A., and de Bruijn J.D. Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. Proceedings of the National Academy of Sciences USA 107, 13614, 2010.
30. Estaca E., Cabezas J., Uson J., Sanchez-Margallo F., Morell E., and Latorre R. Maxillary sinus-floor elevation: an animal model. Clinical Oral Implants Research 19, 1044, 2008.
31. Haas R., Donath K., Fodinger M., and Watzek G. Bovine hydroxyapatite for maxillary sinus grafting: comparative histomorphometric findings in sheep. Clinical Oral Implants Research 9, 107, 1998.
32. Haas R., Mailath G., Dortbudak O., and Watzek G. Bovine hydroxyapatite for maxillary sinus augmentation: analysis of interfacial bond strength of dental implants using pull-out tests. Clinical Oral Implants Research 9, 117, 1998.
33. Jakse N., Tangl S., Gilli R., Berghold A., Lorenzoni M., Eskici A., Haas R., and Pertl C. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clinical Oral Implants Research 14, 578, 2003.
34. Brumund K.T., Graham S.M., Beck K.C., Hoffman E.A., and McLennan G. The effect of maxillary sinus antrostomy size on xenon ventilation in the sheep model. Otolaryngology - Head Neck Surgery 131, 528, 2004.
35. Karabuda C., Arisan V., and Hakan O. Effects of sinus membrane perforations on the success of dental implants placed in the augmented sinus. Journal of Periodontology 77, 1991, 2006.
36. Cameron H.U., Macnab I., and Pilliar R.M. Evaluation of biodegradable ceramic. Journal of Biomedical Materials Research 11, 179, 1977.
37. Szabo G., Huys L., Coulthard P., Maiorana C., Garagiola U., Barabas J., Nemeth Z., Hrabak K., and Suba Z. A prospective multicenter randomized clinical trial of autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevation: histologic and histomorphometric evaluation. International Journal of Oral & Maxillofacial Implants 20, 371, 2005.
38. Knabe C., Koch C., Rack A., and Stiller M. Effect of beta-tricalcium phosphate particles with varying porosity on osteogenesis after sinus floor augmentation in humans. Biomaterials 29, 2249, 2008.
39. Szabo G., Suba Z., Hrabak K., Barabas J., and Nemeth Z. Autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevations (2- and 3-dimensional computed tomographic, histologic, and histomorphometric evaluations): preliminary results. International Journal of Oral & Maxillofacial Implants 16, 681, 2001.
40. Zerbo I.R., Bronckers A.L., de Lange G.L., van Beek G.J., and Burger E.H. (2001) Histology of human alveolar bone regeneration with a porous tricalcium phosphate. A report of two cases.

Clinical Oral Implants Research 12, 379, 2001.

41. Ozyuvaci H., Bilgic B., and Firatli E. Radiologic and histomorphometric evaluation of maxillary sinus grafting with alloplastic graft materials. Journal of Periodontology 74, 909, 2003.
42. Zerbo I.R., Zijderveld S.A., de Boer A., Bronckers A.L., de Lange G., ten Bruggenkate C.M., and Burger E.H. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clinical Oral Implants Research 15, 724, 2004.
43. Zijderveld S.A., Zerbo I.R., van den Bergh J.P., Schulten E.A., and ten Bruggenkate C.M. Maxillary sinus floor augmentation using a beta-tricalcium phosphate (Cerasorb) alone compared to autogenous bone grafts. International Journal of Oral & Maxillofacial Implants 20, 432, 2005.
44. Suba Z., Takacs D., Matusovits D., Barabas J., Fazekas A., and Szabo G. Maxillary sinus floor grafting with beta-tricalcium phosphate in humans: density and microarchitecture of the newly formed bone. Clinical Oral Implants Research 17, 102, 2006.
45. Simunek A., Kopecka D., Somanathan R.V., Pilathadka S., and Brazda T. Deproteinized bovine bone versus beta-tricalcium phosphate in sinus augmentation surgery: a comparative histologic and histomorphometric study. International Journal of Oral & Maxillofacial Implants 23, 935, 2008.
46. Wang S., Zhang Z., Xia L., Zhao J., Sun X., Zhang X., Ye D., Uludag H., and Jiang X. Systematic evaluation of a tissue-engineered bone for maxillary sinus augmentation in large animal canine model. Bone 46, 91, 2010.
47. Jiang X.Q., Sun X.J., Lai H.C., Zhao J., Wang S.Y., and Zhang Z.Y. Maxillary sinus floor elevation using a tissue-engineered bone complex with beta-TCP and BMP-2 gene-modified bMSCs in rabbits. Clinical Oral Implants Research 20, 1333, 2009.
48. Gruber R.M., Ludwig A., Merten H.A., Achilles M., Poehling S., and Schliephake H. Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a histological and histomorphometric study in the Goettingen miniature pig. Clinical Oral Implants Research 19, 522, 2008.
49. Marx R.E., Carlson E.R., Eichstaedt R.M., Schimmele S.R., Strauss J.E., and Georgeff K.R. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics 85, 638, 1998.
50. Park J.B. Use of bone morphogenetic proteins in sinus augmentation procedure. Journal of Craniofacial Surgery 20, 1501, 2009.
51. Plachokova A.S., Nikolidakis D., Mulder J., Jansen J.A., and Creugers N.H. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. Clinical Oral Implants Research 19, 539, 2008.

# SUMMARY
# CLOSING REMARKS
# FUTURE PERSPECTIVES

# SUMMARY AND ADDRESS TO THE AIMS

The general aim of the research described in this thesis was to assess the value of bone substitute materials for maxillofacial bone augmentation procedures as well as to evaluate innovative synthetic bone substitute materials in preclinical bone augmentation models. The general conclusion on the use of bone substitutes for oral and maxillofacial applications is that their performance is predominantly related to bone substitute material properties, recipient site characteristics, and patient conditions.

In bone augmentation procedures for the oral and maxillofacial skeleton, the use of autologous onlay and inlay grafting is still considered the gold standard. Unfortunately, harvesting of such an autologous bone graft is associated with serious drawbacks. The major disadvantage is that donor site surgery requires a prolonged operating time and may cause severe donor site morbidity. Consequently, a broad variety of (synthetic) bone substitute materials have been developed. Clinically relevant advantages of these materials compared to autologous bone include their cost, safety and off-the-shelf availability. In order to improve decision making regarding the augmentation material to be recommended for oral and maxillofacial procedures in individual patients, two meta-analytical studies were performed. Furthermore, patient specific conditions were evaluated for their potential influence on the clinical outcome.

Although a large number of bone substitute materials have been described, research and development in this area is still progressing in order to improve the performance of candidate materials. Three of the most important prerequisites for the clinical acceptance of a novel bone substitute material are excellent perioperative handling properties, degradation over time and replacement by bone tissue in a controlled manner. In view of this, research in this thesis specifically focused on the improvement of clinical handling properties, degradation characteristics and osteoinduction capacity of such materials. The investigated calcium phosphate cements (CPCs) allowed optimal defect filling and easy shaping. Porosity was introduced to enhance material degradation and bone conduction via both a foaming agent ($CO_2$) that forms gas bubbles during CPC setting and several different degradable poly(lactic-co-glycolic acid) (PLGA) microspheres that degrade hydrolytically within the CPC-matrix. CPCs were evaluated in small and large animal models to assess their biological performance and potential for future clinical use. In view of osteoinduction, a novel osteoinductive microstructured calcium phosphate material was assessed in a large animal model.

**Autologous bone grafts and maxillary sinus floor augmentation.**

No studies have been published before that evaluated histomorphometric data from a large number of patients comparing different sites and methods of autologous bone grafting in maxillary sinus floor augmentation procedures. In the **second chapter** of this thesis, a meta-analysis was performed on earlier published data. Pubmed search engine and the following journals were explored: Clinical Oral Implant Research, International Journal of Oral and Maxillofacial Implants, International Journal of Periodontics and Restorative Dentistry, and the Journal of Periodontology. According to the inclusion criteria for this study, 25 out of 147 articles were selected for analysis. The majority were prospective clinical studies, followed by two randomized clinical trials, one pilot and one case series. After statistical analysis, a reference value for Total Bone Volume (TBV) of 47% was found using iliac bone grafting as standard group. The use of intra-oral bone grafts for sinus augmentation increases the TBV; with 11% for chin bone and 14 % for bone grafted from other intra-oral sites. Particulation of a bone graft had a negative effect on TBV of 18%. Surprisingly, no correlation between TBV and the time of graft healing was found. Histological section thickness seemed to be a significant variable, as every micron increase of section thickness lead to an increase of 0.4% of TBV. From the meta-analysis **in chapter 2**, it was concluded that bone grafting from the iliac crest resulted in a significantly lower TBV compared to intra-oral bone grafting. However, due to the limited availability of intra-oral bone to be harvested, iliac grafts still have to be considered as the gold standard in augmenting the severely atrophic maxilla.

**Bone substitute materials and maxillary sinus floor augmentation.**

In addition to the lack of studies comparing the use of autologous bone grafts, no extensive studies have been published before in which histomorphometric data from a large group of patients using various biomaterials for sinus floor augmentation procedures were evaluated. Therefore, in the **third chapter** of this thesis, another meta-analysis was performed. Pubmed and the same journals as in the previous chapter were used for the inclusion of data. According to the inclusion criteria for this study, a total of 64 out of 147 articles were integrated for analysis. Based on autologous bone grafting as gold standard, a reference value for TBV of 63% was found in this study. Particulation of the bone graft also resulted in a general reduction of 18% of TBV. Furthermore, delayed implant placement was found to reduce the TBV with 7%. TBV was 8% or 6% higher, if a biopsy was respectively taken before 4.5 months or after 9.0 months after initial maxillary sinus augmentation surgery. The application of allogenic, xenogenic, or alloplastic bone substitute materials (or combinations thereof) mainly resulted in a significant lower amount of TBV, varying between 7% and 26%, as compared to autologous bone grafting. Inventorying the effect of

'biopsy time' for only autologous bone grafts, showed a TBV which was significantly higher before 4.5 and after 9.0 months of healing time compared to period in between. This effect was not found in the previous meta-analysis. Surprisingly, with respect to 'biopsy time' for bone substitutes other than autologous bone, no significant differences in TBV were found. It was concluded that autologous bone still has to be considered as the gold standard because of higher TBV after maxillary sinus augmentation procedures. However, the consequence of the TBV for dental implant survival still needs to be unraveled.

**Predictive value patient specific characteristics on bone graft resorption.**

As the outcome of a maxillary sinus floor augmentation procedure is based on multiple factors, individual patient characteristics as well as material properties need to be taken in consideration. In the **fourth chapter** of this thesis, it was attempted to identify patient specific parameters to predict maxillary sinus floor augmentation outcome regarding autologous bone graft volume by maxillofacial CT. No studies are available providing predictive radiographic parameters regarding the expected amount of resorption after maxillary sinus augmentation surgery using autologous bone grafts. Therefore, the aim of this study was to determine parameters influencing the outcome of the bone graft resorption process. In 20 patients, three dimensional analysis of alveolar ridge dimensions and bone graft volume change in the atrophic posterior maxilla was performed by Cone Beam Computerized Tomography (CBCT) imaging at several chronological time points. Ridge dimensions were assessed before maxillary sinus augmentation surgery. Bone graft volumes were compared directly after maxillary sinus floor augmentation surgery and after a variable graft healing interval. To analyze the relation between bone volume change with the independent variables patients gender, age, alveolar crest height and width, and graft healing time interval, a multi-level extension of linear regression was applied. A residual bone height of 6.0 mm (SD=3.6 mm) and 6.2 mm (SD=3.6 mm) was found for the left and right sides, respectively. Moreover, alveolar bone widths of 6.5 mm (SD=2.2 mm) and 7.0 mm (SD=2.3 mm) at the premolars and 8.8 mm (SD=2.2 mm) and 8.9 mm (SD=2.5 mm) at the molars regions were found for the left and right side, respectively. Bone graft volume decreased on average 25.0% (SD=21.0%) after an average healing interval of 4.7 months (SD=2.7, median=4.0 months). The variables 'age' ($p$=0.009) and mean alveolar crest 'bone height' ($p$=0.043), were identified as significant regarding bone graft resorption. A decrease of 1.0% (SE=0.3%) bone graft resorption was found for each year the patient gets older and an increase in bone graft resorption of 1.8% (SE=0.8%) was found for each mm of original bone height before maxillary sinus floor augmentation. It was concluded that graft resorption occurs when using autologous bone grafts for maxillary sinus augmentation and that alveolar crest

bone height and patient age have a significant effect on graft resorption, with increased resorption for higher alveolar crest bone height and decreased resorption for older patients. Consequently, patient characteristics that affect the process of bone graft resorption should be given full consideration when performing maxillary sinus augmentation surgery.

**Three porous calcium phosphate cements for bone augmentation.**

Pre-implant surgery has become a routine procedure to obtain sufficient bone quantity and quality for dental implant installation in patients with initially inadequate bone volumes. Although autologous bone onlay or inlay grafting is still the preferred bone augmentation technique, a broad range of synthetic bone substitutes have been developed, such as calcium phosphate cement (CPC). The introduction of porosity within CPC can be used to increase CPC degradation and bone ingrowth. Therefore, in **chapter 5** three different strategies to obtain porous CPCs and their performance were evaluated in a preclinical augmentation model. Instantaneously porous CPC (CPC-IP) was compared to delayed porous CPC *in vitro* and *in vivo*. CPC-IP was obtained by creation of $CO_2$ bubbles during setting, whereas delayed porous CPC was obtained after degradation of incorporated PLGA microspheres. As an additional aspect, delayed porous CPC was created by the incorporation of either hollow or dense degradable PLGA microspheres (CPC-hPLGA and CPC-dPLGA). All CPC compositions showed appropriate clinical handling properties and an interconnected porous structure with a final porosity above 70%. *In vitro* degradation studies showed gradual formation of pores and further CPC-matrix dissolution for CPCs containing PLGA microspheres (dense PLGA microspheres > hollow PLGA microspheres), whereas hardly any degradation was observed for CPC-IP. For *in vivo* evaluation of the CPCs, an augmentation model was used, allowing CPC injection into a rigidly immobilized Teflon ring on the rat skull. Histological evaluation after 12 weeks of implantation showed bone formation using all three CPCs. Bone apposition reached volumetric amounts of up to 10% of the augmentation area and a maximum augmentation height of ~1 mm. CPC-IP showed significantly more bone formation and resulted in a superior bone apposition height compared to both CPCs containing PLGA microspheres. No differences in biological performance were observed between CPCs containing hollow or dense PLGA microspheres.

**Maxillary sinus floor augmentation with CPC-PLGA composites in sheep.**

The aim of the preclinical study described in **chapter 6** was to evaluate the biological performance of two injectable CPC composite materials containing PLGA microspheres with different properties in a maxillary sinus floor elevation model in sheep. PLGA microspheres were made of either low molecular weight (LMW; ~17 kDa) acid-terminated PLGA ($PLGA_{L-AT}$) or high molecular weight

(HMW; ~44 kDa) end-capped PLGA ($PLGA_{H\text{-}EC}$) and incorporated in CPC. Eight female Swifter sheep underwent a bilateral maxillary sinus floor elevation procedure via an extra-oral approach. All animals received both materials, alternately injected in the left and right sinus cavity (split-mouth model) and a healing period of 12 weeks was used. Analysis of biological performance was based on histology, histomorphometry and evaluation of sequential fluorochrome labeling. Both types of CPC-PLGA composites showed biocompatibility and direct bone-cement contact. CPC-$PLGA_{L\text{-}AT}$ showed a significantly higher degradation distance compared to CPC-$PLGA_{H\text{-}EC}$ (1949 µm ± 1295 µm versus 459 µm ± 267 µm; $p<0.05$). Further, CPC-$PLGA_{L\text{-}AT}$ showed significantly more bone in the region of interest (26.4% ± 10.5% versus 8.6% ± 3.9%; $p<0.001$) and significantly less remaining CPC material (61.2% ± 17.7% versus 81.9% ± 10.9%; $p<0.05$). It was concluded that both CPC-$PLGA_{L\text{-}AT}$ and CPC-$PLGA_{H\text{-}EC}$ are safe materials for sinus floor elevation procedures in a large animal model, presenting biocompatibility and direct bone contact. In view of material performance, CPC-$PLGA_{L\text{-}AT}$ showed significantly faster degradation and a significantly higher amount of newly formed bone compared to CPC-$PLGA_{H\text{-}EC}$.

**Maxillary sinus floor augmentation with osteoinductive ceramic in sheep.**

The objective of the study described in **chapter 7**, was to evaluate the biological performance of novel osteoinductive micro-structured tricalcium phosphate (MSTCP) particles in maxillary sinus floor augmentation surgery in sheep. For this purpose, maxillary sinus floor augmentation with MSTCP particles was unilaterally performed in eight Swifter sheep. Radiological imaging and histological analyses were performed after 12 weeks of implantation. Maxillofacial CT, histology, histomorphometrical analysis, and sequential polychrome fluorescent labeling indicated that MSTCP particles provided an excellent scaffold for cell ingrowth and bone formation. After a 12 week implantation period, the augmented sinuses showed an increased bone height of ~6 mm and a mean total bone volume of 43% with significant degradation of the implanted MSTCP particles. Consequently, it was concluded that MSTCP particles represent a suitable bone substitute material for maxillary sinus floor augmentation surgery.

# CLOSING REMARKS

In this thesis, research efforts focused on the use of autologous bone and bone substitute materials for maxillofacial bone augmentation procedures. Two meta-analytical studies and a trohoc study provided an overview of the current concepts in the development of these augmentation materials and their biological performance. It could be concluded that in reconstructive bone augmentation procedures of the maxillofacial skeleton, autologous bone grafts must still be considered the gold standard. Furthermore, it was concluded that the biological outcome of augmentation procedures is related to augmentation material properties, the applied surgical technique and patient conditions. Recent literature, however, evaluating the clinical outcome after applying various bone substitute materials versus autologous bone could not identify significant advantages of using the one or the other. In view of this, the consequence of the biological performance on final dental implant survival is still unraveled. It is questionable if histology and histomorphometry, which are considered the standard tools for the assessment of the biological performance of bone augmentation materials, provide sufficient information for adequate decision making. In addition, also alveolar crest bone height and patient age showed to be of significant influence on autologous bone graft resorption. An increased resorption was found for higher alveolar crest bone height and a decreased resorption for older patients. Thus, beside the augmentation material point of view also patient characteristics and the clinical outcome must be given full consideration when performing and assessing maxillary sinus floor augmentation surgery.

In spite of these patient specific factors, an increasing number of bone substitute materials is becoming (commercially) available for clinical use. The collaboration of experts from different scientific fields has resulted in an explosive growth of material based possibilities for bone reconstructive procedures. Such synthetic bone substitute materials have the general advantage of off-the-shelf availability and reduction of patient morbidity. Moreover, these bone substitute materials have highly standardized properties and allow production in relatively large amounts with a consequential predictable clinical outcome. Novel concepts for further innovation are based on the combination of scientific knowledge and clinical needs. Nowadays, especially improvement of clinical handling and bone regenerative properties bear high attention.

Considering handling properties, CPCs from this study exhibited a high potential because of easy shaping and optimal defect filling. From a biological point of view, the introduction of porosity resulted in an interconnected porous

structure with sufficient macroporosity for bone ingrowth within a controllable period after injection. Moreover, the investigated CPCs showed high biocompatibility as these did not elicit any adverse reactions upon implantation in a bony environment. However, a major disadvantage is that these CPCs are solely osteoconductive. While the inclusion of biologically active factors to obtain osteoinductive capacity is an expensive treatment option, the innovation of material based osteoinductivity overcomes this disadvantage. In view of this, the microstructured TCP granules used in this study possess intrinsic osteoinductive capacity and excellent biological performance. On the other hand, handling properties of this material have been shown to be inferior to CPCs. Therefore, further research and development of synthetic materials for bone reconstructive procedures should continue. The possibilities to design new materials are numerous and a small modification of chemical properties might substantially affect the biological performance of such a bone substitute material.

From a clinical point of view, clinicians should have the possibility to select the most appropriate augmentation material for each specific clinical situation. However, an important issue that needs to be addressed is the fact that despite the clinicians aim for the most optimal treatment for a given patient, reality proofs that not always the most suitable bone substitute material is selected. A wide range of options is available, however clinicians only have experience with limited types of materials. Furthermore, (private) medical centers are usually loyal to the company that supplied such materials in the past and the costs of new materials are usually higher. Moreover, due to the rapidly evolving development of augmentation materials, clinicians are not always aware of the existence and advantages of innovative bone graft substitute materials compared to familiar equivalents.

# FUTURE PERSPECTIVES

Although this thesis revealed relevant data on the use and development of bone substitute materials for augmentation purposes in the oral and maxillofacial area, several aspects need further investigation:

-The primary outcome of the assessment of biological performance of bone substitute materials is defined as the histological and histomorphometrical findings of biopsies taken before implant placement. However, the outcome and consequence of such measurement on implant survival are still not completely unraveled yet and need to be further investigated.

-The healing periods elapsed after maxillary sinus floor augmentation procedures in different studies varied to a great extent. Although this is an important factor in patient treatment, only for autologous bone a significant trend was found in one of the two meta-analytical studies. Therefore, further research should focus on determining ultimate graft healing time intervals for each specific bone substitute materials.

-The height of the alveolar ridge was determined by CBCT to be of significant influence on autologous bone graft resorption. However, in most studies the height of the alveolar ridge was only considered as the base for the decision of immediate or delayed implant placement. In view of this, also the influence of the alveolar ridge on the biological performance of bone substitute materials should be further investigated.

-From this study it is not possible to decide whether general diseases, smoking or other risk factors have an influence on the biological performance of bone augmentation materials. Because these aspects becomes more and more important in future medicine, further studies should assess these possible influences.

-Further research is necessary to specifically tailor the degradation rate of incorporated PLGA microspheres and CPC itself in order to allow gradual bone ingrowth and material degradation over time for specific clinical applications.

-The influence of biologically active factors to promote bone formation, or even better material based intrinsic osteoinductive capacity needs further investigation in order to perform more complicated augmentation procedures or to use such materials in patients with a compromised bone regeneration potential.

-Considering the experiments as performed and knowledge as obtained from this thesis, it has to be recommended that clinical trials are initiated to investigate the safety and efficacy of osteoinductive microstructured TCP granules and (porous) calcium phosphate cements. Moreover, a maxillary sinus augmentation study is advisable as preclinical studies showed promising results compared to other bone augmentation procedures.

# SAMENVATTING

# SAMENVATTING EN EVALUATIE VAN DE DOELSTELLINGEN

Het doel van het onderzoek, dat is beschreven in dit proefschrift, was om het gebruik van botvervangende materialen voor maxillofaciale botaugmentatie procedures te evalueren. Daarnaast werden innovatieve synthetische botvervangende materialen in preklinische modellen getest. In het algemeen kon worden geconcludeerd, dat de werking van botvervangende materialen in kaak- en aangezichtschirurgische procedures voornamelijk is gerelateerd aan de eigenschappen van het materiaal, de omgeving waarin het materiaal wordt aangebracht maar ook aan de individuele condities van de patiënt.

Tegenwoordig wordt het autoloog bottransplantaat beschouwd als de gouden standaard in botaugmentatie procedures in het kaak en aangezichtsskelet. Het oogsten van een dergelijk bottransplantaat is echter geassocieerd met aanzienlijke nadelen. Het grootste bezwaar is de verlengde operatieduur als gevolg van de operatie aan de donorsite en de mogelijk ernstige donorsite morbiditeit die hiermee gepaard gaat. Er zijn daarom reeds een grote hoeveelheid (synthetische) bot vervangende materialen ontwikkeld. Klinische voordelen in vergelijking met een autoloog bottransplantaat zijn de kosten, veiligheid en directe en ongelimiteerde beschikbaarheid. In dit proefschrift werden een tweetal meta-analyses verricht om inzicht te verwerven in de keuzemogelijkheden voor wat betreft het augmentatiemateriaal voor sinusbodemelevatie procedure in individuele patiënten. Daarnaast werd de mogelijke invloed van patiënt specifieke condities op de klinische uitkomst onderzocht middels een driedimensionale röntgenstudie.

Onderzoek en ontwikkeling op het gebied van botvervangende materialen continueert zich en is gericht op het verbeteren van materiaaleigenschappen. Goede klinische toepasbaarheid van een botvervangend materiaal is afhankelijk van drie factoren. Voor succesvol gebruik zijn excellente peri-operatieve hanteringeigenschappen, degradatie in de tijd en gecontroleerde vervanging door bot nodig. Het onderzoek, dat is beschreven in dit proefschrift, richt zich daarom ook op het verbeteren van de hanteringeigenschappen, degradatie-karakteristieken en het osteoinductieve karakter van botvervangende materialen. Er werd onderzoek gedaan naar calcium fosfaat cement (CPC), dat bot-defecten op een optimale wijze kan vullen en gemakkelijk is te vervormen. Porositeit van het CPC werd geïnduceerd om de degradatie en osteoconductive eigenschappen te verbeteren. Zowel het invangen van $CO_2$ bubbels tijdens het uitharden van het CPC, als de toevoeging van verschillende oplosbare poly (lactic-co-glycolic acid) (PLGA) microsferen werd onderzocht. De biologische

prestaties en het mogelijk klinische gebruik in de toekomst van dit materiaal werden zowel in kleine als grote diermodellen onderzocht. In het kader van osteoinductie werd een osteoinductief microstructured calcium fosfaat materiaal onderzocht in een groot diermodel.

**Autologe bottransplantaten voor sinusbodemelevatie procedures**

Tot op heden zijn er geen studies gepubliceerd waarin de histomorfometrische uitkomst is vergeleken van verschillende donorsites en methodes van autologe bottransplantatie na sinusbodemelevatie bij een groot aantal patiënten. In het **tweede hoofdstuk** van dit proefschrift werd daarom een meta-analyse verricht van de in de literatuur gepubliceerde data. Pubmed en de volgende tijdschriften werden handmatige doorzocht: Clinical Oral Implants Research, International Journal of Oral and Maxillofacial Implants, International Journal of Periodontics and Restorative Dentistry en het Journal of Periodontology. Volgens de inclusie-criteria van deze studie werden 25 van de 147 gevonden artikelen gebruikt voor meta-analyse. Het gros van deze publicaties beschreef prospectieve klinische studies, waarbij ook een tweetal gerandomiseerde prospectieve studies, één pilotstudie en één case serie werden gevonden. Na de statische analyse werd een referentiewaarde voor Totaal Bot Volume (TBV) van 47% gevonden. Als standaardgroep voor deze analyse werd bot uit de crista iliaca genomen. Sinusbodemelevatie met intra-oraal verkregen bot resulteerde in een hogere TBV; 11% met kinbot en 14% met bot van een andere intra-orale donorsite. Er werd, onverwacht, geen correlatie gevonden tussen TBV en de inhelingstijd van het bottransplantaat. Histologische sectiedikte was wel een significante variabele, elke micrometer toename in dikte resulteert in 0.4% toename van het TBV. Uit de meta-analyse in **hoofdstuk 2** kon daarom worden geconcludeerd dat bottransplantatie met bot uit de crista iliaca resulteert in een significant lagere TBV in vergelijking met bottransplantaties van een intra-orale donorsite. Echter, omdat de hoeveelheid intra-oraal bot dat geoogst kan worden relatief klein is, worden bottransplantaten uit de crista iliaca nog steeds als de gouden standaard beschouwd voor sinusbodemelevatie procedures in de ernstig atrofische maxilla.

**Botvervangende materialen voor sinusbodemelevatie procedures**

Naast studies voor autologe bottransplantaten zijn er ook geen uitgebreide studies naar botvervangende materialen bij grote groepen patiënten gedaan. Daarom werd in het **derde hoofdstuk** van dit proefschrift een tweede meta-analytische studie verricht. Pubmed en dezelfde tijdschriften als in het voorgaande hoofdstuk werden doorzocht. Volgens de inclusiecriteria bij deze studie werden 64 van de 147 gevonden publicaties gebruikt voor de meta-analyse. Een referentiewaarde van 63% werd berekend voor TBV op basis van autoloog bot als standaardgroep. Het gebruik van partikels verlaagt het TBV

met 18% ten opzicht van bloktransplantaten. Verder werd een 7% lager TBV gevonden wanneer tandheelkundige implantaten in twee fasen werden geplaatst. TBV was 8% of respectievelijk 6% hoger als een biopt binnen 4.5 maand of na 9.0 maanden na sinusbodemelevatie werd genomen. Het gebruik van allogene, xenogene of alloplastische botvervangende materialen (of combinaties daarvan) resulteerde in het algemeen in een significant lager TBV, variërend tussen 7% en 26% vergeleken met autologe bottransplantaties. Het effect van 'bioptie tijd' liet een significant hoger TBV zien voor 4.5 en na 9.0 maanden of inhelingstijd vergeleken met de tussenliggende periode voor alleen autologe bottransplantaten. Dit effect werd niet gezien in de meta-analyse van het voorgaande hoofdstuk. Verrassend genoeg werd er geen significant effect van 'bioptie tijd' op het TBV gevonden bij de verschillende botvervangende materialen. Concluderend kon worden gesteld dat autoloog bot nog steeds als de gouden standaard moet worden beschouwd voor sinusbodemelevatie procedures vanwege het hogere TBV. Nochtans zijn de consequenties van dit hoge of lagere TBV op tandheelkundige implantaatoverleving nog niet geheel duidelijk.

**Voorspellende waarde van patiënt specifieke karakteristieken op bottransplantaat resorptie.**

De uitkomst van een sinusbodemelevatie procedure is afhankelijk van verschillende factoren, dit zijn onder andere materiaaleigenschappen maar ook individuele patiënt karakteristieken. In het **vierde hoofdstuk** van dit proefschrift werd getracht middels maxillofaciale CT patiënt specifieke factoren te identificeren die een voorspellende waarde hebben op bottransplantaat resorptie na sinusbodemelevatie procedures. Er zijn in de literatuur geen studies beschikbaar die dergelijke voorspellende radiologische parameters beschrijven. Daarom was het doel, van deze studie in dit hoofdstuk, om verschillende parameters te onderzoeken op hun mogelijke invloed. In totaal werd bij twintig patiënten op drie tijdspunten een driedimensionale analyse verricht van alveolaire botdimensies en bottransplantaatvolume na sinusbodemelevatie middels Cone Beam Computerized Tomography (CBCT). Alveolair botdimensies werden gemeten voorafgaand aan de sinusbodemelevatie procedure. Bottransplantaatvolume werd gemeten direct na sinusbodemelevatie en na een variabele inhelingstijd. Een multi-level extensie van regressie analyse werd verricht om de invloed van de variabelen: geslacht, leeftijd, alveolaire bothoogte en breedte en inhelingstijd te analyseren. Een residuele alveolaire bothoogte van 6.0 mm (SD=2.2 mm) en 6.2 mm (SD=3.6 mm) werd gemeten voor de respectievelijk linker- en rechterkant. Verder werd een alveolaire botbreedte van 6.5 mm (SD=2.2 mm) en 7.0 mm (SD=2.3 mm) ter hoogte van de premolaren en 8.8 mm (SD=2.2 mm) en 8.9 mm (SD=2.5 mm) ter hoogte van de molaar regio

gevonden voor respectievelijk links en rechts. Bottransplantaatvolume verminderde met 25% (SD=21%) na een gemiddelde inhelingstijd van 4.7 maanden (SD=2.7 maanden, mediaan=4.0 maanden). De variabelen 'leeftijd' ($p$=0.009) en gemiddelde 'alveolaire bothoogte' ($p$=0.043) bleken van significante invloed op deze bottransplantaat resorptie. Een volume verhoging van 1.0% (SE=0.3%) werd gevonden voor elk jaar dat de patiënt ouder was en een volume afname van 1.8% (SE=0.8%) werd gevonden voor elke pre-existente millimeter alveolaire bothoogte voorafgaand aan de ingreep. Er kon worden geconcludeerd, dat bottransplantaat resorptie optreedt na sinusbodemelevatie procedures en dat de leeftijd van de patiënt en de pre-existente bothoogte een significante invloed hebben op de mate van resorptie. Met een toename van resorptie bij hogere pre-existente bothoogtes en een verminderde resorptie bij een hogere leeftijd. Patiënt karakteristieken waarvan in studieverband is aangetoond dat ze een significante invloed hebben op bottransplantaat resorptie moeten daarom ook alle aandacht krijgen wanneer een sinusbodemelevatie wordt verricht bij de individuele patiënt.

**Drie poreuze calcium fosfaat cementen voor bot augmentatie.**

Pre-implantologische chirurgie is een routinematige procedure voor patiënten met een initieel tekort aan bot voor tandheelkundige implantaatplaatsing. Alhoewel het autologe bottransplantaat wordt beschouwd als de gouden standaard, is een groot aantal synthetische botvervangende materialen ontwikkeld. Een voorbeeld hiervan is calcium fosfaat cement (CPC). Het creëren van porositeit in dit CPC draagt bij aan een verbeterde degradatie met als gevolg een verhoogde mate van botingroei in het materiaal. Daarom zijn in **hoofdstuk 5** van dit proefschrift een drietal methoden geëvalueerd om porositeit te introduceren in het CPC en getest in een preklinisch augmentatie model. Direct poreus CPC (CPC-IP) werd zowel *in vitro* als *in vivo* vergeleken met vertraagd poreus CPC CPC-IP. CPC-IP werd verkregen door het invangen van vrijgekomen $CO_2$ bubbels tijdens het uitharden van het CPC. Vertraagd poreus CPC werd verkregen door het toevoegen van degradeerbare PLGA microsferen aan het CPC poeder. Additioneel aspect was het gebruik van zowel holle als van volledige gevulde PLGA microsferen (respectievelijk CPC-hPLGA en CPC-dPLGA). Alle drie de CPC composities lieten goede hanteringeigenschappen en een inter-geconnecteerde poreuze structuur zien met een totale porositeit van boven de 70%. De *in vitro* degradatiestudie liet een graduele formatie van poriën zien en een toegenomen CPC-matrix ontbinding in het CPCs met PLGA microsferen (dPLGA microsferen > hPLGA microsferen), terwijl er nagenoeg geen degradatie werd geobserveerd in CPC-IP. Voor de *in vivo* evaluatie van de CPCs werd gebruik gemaakt van een augmentatie model in ratten. CPCs werden geïnjecteerd in een Teflon ring die stevig op de schedel van deze ratten was

geïmmobiliseerd. Histologisch evaluatie na een inhelingstijd van 12 weken liet botformatie zien in alle drie de CPCs. Het botaugmentatievolume bereikte 10% van het augmentatiegebied met een maximaal gemiddelde augmentatiehoogte van 1 mm. Het gebruik van CPC-IP resulteerde in een significant grotere hoeveelheid bot en in een superieure botappositiehoogte vergeleken met beide CPCs met PLGA microsferen. Daarbij werd er geen significant verschil gevonden tussen het gebruik van dPLGA of hPLGA microsferen.

**Sinusbodemelevatie met CPC-PLGA composieten in schapen**

Het doel van de preklinische studie, die werd beschreven in **hoofdstuk 6** van dit proefschrift, was om de biologisch prestaties van twee injecteerbare CPC-PLGA microsfeer composieten te evalueren in een sinusbodemelevatie model in schapen. De PLGA microsferen werden gemaakt van een laag moleculairgewicht PLGA (LMW; ~17 kDa) acid-terminated PLGA ($PLGA_{L\text{-}AT}$) of van een hoog moleculairgewicht PLGA (HMW; ~44 kDa) end-capped PLGA ($PLGA_{H\text{-}EC}$). Deze microsferen werden, net als in het vorige hoofdstuk, geïncorporeerd in het CPC poeder. Acht vrouwelijke Swifter schapen ondergingen een bilaterale sinusbodemelevatie procedure via een extra-orale benadering. CPCs werden volgens een split-mouth model geïnjecteerd in de sinus maxillaris en er werd een inhelingstijd van 12 maanden in acht genomen. De analyse van de biologische prestaties werd verricht middels histologie, histomorfometrie en evaluatie van sequentieel toegevoegde fluorochromen. Beide typen CPC-PLGA microsfeer composieten lieten een adequate biocompatibiliteit en een direct bot-cement contact zien. CPC-$PLGA_{L\text{-}AT}$ resulteerde in een grotere degradatie-afstand vergeleken met CPC-$PLGA_{H\text{-}EC}$ (1949 µm ± 1295 µm versus 459 µm ± 267 µm; $p<0.05$). Verder liet CPC-$PLGA_{L\text{-}AT}$ een grotere hoeveelheid bot in het geselecteerde gebied van interesse zien (26.4% ± 10.5% versus 8.6% ± 3.9%; $p<0.001$) en significant minder residuaal CPC materiaal (61.2% ± 17.7% versus 81.9% ± 10.9%; $p<0.05$). Er kon worden geconcludeerd, dat zowel CPC-$PLGA_{L\text{-}AT}$ als CPC-$PLGA_{H\text{-}EC}$ beide veilige materialen zijn voor sinusbodemelevatie in een groot diermodel met een adequate biocompatibiliteit en direct botcontact. Echter vanuit het materiaaloogpunt liet CPC-$PLGA_{L\text{-}AT}$ een significant snellere degradatie en een significant hogere hoeveelheid nieuw gevormd bot zien vergeleken met CPC-$PLGA_{H\text{-}EC}$.

**Sinusbodemelevatie met een osteoinductief keramiek in schapen.**

Het doel van de studie, beschreven in **hoofdstuk 7**, was om de biologische prestaties van een osteoinductief micro-structured tricalcium fosfaat (MSTCP) keramiek te evalueren voor sinusbodemelevatie procedures in schapen. Om dit doel te bereiken werd er een unilaterale sinusbodemelevatie uitgevoerd met MSTCP partikels in acht Swifter schapen. Radiologische beeldvorming en histologische analyse werd uitgevoerd na een inhelingstijd van 12 weken.

Maxillofaciale CT, histologie, histomorfometrie en sequentieel toegevoegde fluorochromen lieten zien dat MSTCP partikels een excellente scaffold bieden voor celingroei en botformatie. 12 weken na sinusbodemelevatie werd een sinusbodem verhoging van ongeveer 6 mm en een gemiddeld totaal botvolume van 43% gezien met daarbij significante degradatie van de geïmplanteerde MSTCP partikels. Het kon daarom worden geconcludeerd, dat het onderzochte MSTCP keramiek een geschikt botvervangend materiaal is voor sinusbodemelevatie procedures.

## SLOTOPMERKINGEN

Het onderzoek, dat in dit proefschrift werd beschreven, richt zich op autologe bottransplantaten en botvervangende materialen voor botaugmentatie procedures in kaak- en aangezichtschirurgische toepassingen. Er werd een tweetal meta-analytische en een trohoc studie verricht die inzicht hebben gegeven in de huidige concepten, ontwikkelingen en biologische prestaties van dergelijke materialen. Op basis van deze studies kon worden geconcludeerd, dat het autologe bottransplantaat moeten worden beschouwd als de gouden standaard voor botaugmentatie procedures van het kaak- en aangezichtsskelet. Daarbij werd geconcludeerd, dat de biologische uitkomst na een augmentatie procedure afhankelijk is van augmentatiemateriaal eigenschappen, de chirurgische techniek en verschillende patiënt specifieke condities. Recente literatuur waarin uitsluitend de klinische uitkomst van verschillende botvervangende materialen voor sinusbodemelevatie werd vergeleken ten opzichte van het autologe bottransplantaat liet echter zien, dat er geen specifiek voordeel is voor één van deze therapieën. Er moet daarom worden opgemerkt, dat de relatie tussen de uitsluitend biologische uitkomst na het gebruik van een botaugmentatie materiaal en de tandheelkundige implantaat overleving nog niet geheel duidelijk is. Verder moet men zich afvragen of de standaard evaluatiemethoden, namelijk de histologie en histomorfometrie, voldoende informatie bieden om een adequate keus voor een augmentatie-materiaal te kunnen maken. Bovendien liet het onderzoek in dit proefschrift ook zien, dat de alveolaire bothoogte en de leeftijd van de individuele patiënt een significante invloed hebben op de resorptie van een bottransplantaat. Er werd meer resorptie van het bottransplantaat gezien bij een hogere residuele alveolaire bothoogte en een verlaagde resorptie in oudere patiënten. Op basis van deze bevindingen moet daarom worden geconcludeerd, dat de keuze voor een specifiek augmentatiemateriaal niet alleen af moet hangen van materiaal-eigenschappen maar ook van individuele patiëntkarakteristieken die de klinische uitkomst beïnvloeden.

Er komt een groeiend aantal (commercieel) verkrijgbare bot vervangende materialen voor klinische gebruik op de markt. De intensieve samenwerking tussen experts op verschillende wetenschappelijke gebieden resulteerde in een explosieve groei van mogelijkheden tot het aanpassen van materiaaleigenschappen voor botreconstructieve toepassingen. Synthetische botvervangers hebben het algemene voordeel dat ze direct beschikbaar en veilig zijn. Daarnaast is een belangrijk voordeel de reductie van de mogelijke morbiditeit zoals dit wordt gezien bij het oogsten van een autoloog bottransplantaat. Verder hebben deze botvervangende materialen gestandaardiseerde eigenschappen waardoor ze in een grote hoeveelheid kunnen worden geproduceerd en een voorspelbare en betrouwbare klinische uitkomst bieden. Innovatieve concepten voor de verbetering van deze materialen komt voort uit een combinatie tussen wetenschappelijke vooruitgang en behoeften vanuit de klinische praktijk. Tegenwoordig richt deze innovatie zich vooral op klinische hanteerbaarheid en het verbeteren van de botregeneratieve capaciteit.

Calcium fosfaat cement (CPC) heeft het voordeel, dat het materiaal zich gemakkelijk laat vormen en een optimale defectvulling geeft resulterend in een uitstekende hanteerbaarheid. De introductie van porositeit in het CPC draagt bij aan een geinterconnecteerde macroporeuze structuur voor gecontroleerde botingroei. Verder lieten de CPCs, die in de preklinische diermodellen zijn onderzocht, een goede biocompatibiliteit zien. Een beperking van de onderzochte CPCs is, dat zij uitsluitend een osteoconductief karakter hebben. Daarbij is het zo, dat het incorporeren van biologisch actieve stoffen zoals groeifactoren kostbaar is. De ontwikkeling van osteoinductieve materialen voorkomt dit probleem. De onderzochte microstructured TCP granules hebben een reeds bewezen osteoinductief karakter en het gebruik resulteerde in een excellente biologische prestatie. Daarentegen zijn dergelijke granules minder goed te hanteren in een complexe klinische toepassing dan CPC. Onderzoek en ontwikkeling op het gebied van botregeneratieve materialen zal verder gaan. De mogelijkheden tot het ontwikkelen van botvervangende materialen zijn omvangrijk en het is reeds aangetoond dat kleine veranderingen van bijvoorbeeld chemische eigenschappen een substantieel effect op de biologisch prestaties kunnen hebben. Clinici hebben een uitgebreide keuze uit diverse augmentatiematerialen voor iedere individuele procedure. Hierbij moet echter worden benadrukt, dat de realiteit vaak anders is en niet altijd het meest geschikte materiaal wordt gekozen voor de individuele patiënt. De expertise en ervaring van clinici beperkt zich vaak tot een geselecteerde hoeveelheid materialen en daarnaast zijn medische instellingen vaak loyaal aan één bepaalde fabrikant. Verder is de kostprijs van nieuwe materialen vaak hoger. Bovendien blijkt, dat de snelle ontwikkelingen op het gebied van botvervangende materialen niet door iedereen bij te houden is en dat clinici vaak niet op de hoogte zijn van de voordelen van dergelijke innovatieve materialen.

# TOEKOMSTPERSPECTIEVEN

In dit proefschrift worden de bevindingen beschreven uit het onderzoek naar het gebruik en de ontwikkeling van botvervangende materialen voor botaugmentatie procedures in het mond-, kaak- en aangezichtsgebied. Voortbordurend op deze resultaten zijn er nog een aantal aanvullende punten die kunnen leiden tot vervolgonderzoek.

- De primaire uitkomst van het onderzoek naar de biologisch prestaties van botvervangende materialen wordt bepaald door histologische en histomorfometrische metingen aan het biopt dat is genomen voordat tandheelkundige implantaten worden ingebracht. De invloed en consequenties van deze uitkomst op de overleving van tandheelkundige implantaten is echter nog niet geheel ontrafeld en dienen daarom verder te worden onderzocht.

- De inhelingstijd na een sinusbodemelevatie procedure wordt beschouwd als een belangrijk factor op de klinische uitkomst. Ondanks de grote variatie in de literatuur werd alleen voor autologe bottransplantaten een significante trend gevonden in één van de twee meta-analytische studies. Er kan daarom verder onderzoek worden verricht naar de optimale inhelingstijd voor individuele botvervangende materialen.

- De hoogte van de alveolair botrand bleek van significante invloed op de resorptie van het autologe bottransplantaat na een sinusbodemelevatie procedure. In de literatuur wordt deze waarde slechts gebruikt om een keuze te maken tot directe of vertraagde tandheelkundige implantaat plaatsing. In het licht van deze bevinding is het een vervolgonderzoek aan te raden naar de invloed van deze alveolaire bothoogte op de biologische prestaties van botvervangende materialen.

- Op basis van studies beschreven in dit proefschrift kan niet worden geconcludeerd of (systeem)ziekten, roken of andere risicofactoren een invloed hebben op de biologische prestaties van botervervangende materialen. Omdat deze aspecten steeds belangrijker worden in de geneeskunde zal hier verder onderzoek nodig zijn.

- Vervolgonderzoek is noodzakelijk om verder inzicht en sturing te geven aan de degradatie eigenschappen van in CPC geïncorporeerde PLGA microsferen. Op deze wijze kan botingroei en materiaal degradatie gedurende de inhelingstijd beter worden afgestemd op een individuele klinische situatie.

- De invloed van biologisch actieve factoren om botaanmaak te stimuleren, of nog beter, materiaal dat een intrinsieke osteoinductieve capaciteit heeft, zal verder moeten worden ontwikkeld om succesvolle botaugmentatie procedures in patiënten met een gecompromitteerde botregeneratieve capaciteit mogelijk te maken.

- Het onderzoek en de resultaten daarvan, zoals in dit proefschrift beschreven, geven aanleiding tot het opzetten van klinische studies waarin zowel de osteoinductieve TCP granules als het (poreus) CPC op veiligheid en doelmatigheid worden geëvalueerd. De sinusbodemelevatie procedure kan hiervoor worden aangeraden.

# ACKNOWLEDGEMENTS (DANKWOORD)

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# CURRICULUM VITAE

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Reinder Jan Klijn (roepnaam Reinoud) werd op 6 april 1984 geboren te Callantsoog. Na het behalen van zijn VWO diploma in 2002 aan de Openbare Scholen Gemeenschap te Schagen werkte hij gedurende één jaar als horecamedewerker in het Golden Tullip hotel in Callantsoog. Na dit jaar van oriëntatie begon hij in 2003 aan de nieuwe zesjarige opleiding Technische Geneeskunde aan de Universiteit Twente. Zowel de Bachelor- als Masterfase van deze opleiding werden nominaal doorlopen. Al vroeg tijdens de studie werd zijn belangstelling gewekt voor de reconstructieve en regeneratieve geneeskunde. Zijn klinische stages op dit deelgebied van de Technisch Geneeskunde deed hij in de drie verschillende ziekenhuizen van Nijmegen. Zijn eerste stage was op de afdeling Mondziekten, Kaak- en Aangezichtschirurgie, daarna volgde stages op de afdelingen Bloedtransfusie en Transplantatie Immunologie, Neurochirurgie en Orthopedie. In het zesde jaar van zijn studie participeerde hij in een onderzoeksproject op de afdeling Parodontologie en Biomaterialen van het UMC St Radboud te Nijmegen onder begeleiding van prof. dr. Gert Meijer en drs. Jan Willem Hoekstra. Dit afstudeeronderzoek was de eerste aanzet voor het promotietraject waarvan de resultaten in dit proefschrift zijn beschreven. Het promotieonderzoek werd uitgevoerd tussen 2009 en 2012 (promotoren prof. dr. Gert Meijer en prof. dr. John Jansen). De resultaten van het onderzoek werden reeds op nationale en internationale congressen gepresenteerd. Na het afronden van de opleiding Technische Geneeskunde werd Reinoud in september 2009 tevens toegelaten tot de studie Geneeskunde aan de Radboud Universiteit te Nijmegen. In 2011 behaalde hij zijn Bachelorexamen, waarna hij direct startte met zijn coschappen in diverse aan het UMC St Radboud geaffilieerde instellingen.

Een groot deel van zijn Enschedese studententijd woonde hij op studentenhuis Huize Probitas. Hij was bestuurslid van studievereniging Paradoks en hield zich bezig met verschillende onderwijsgerelateerde zaken. Hij was vice-voorzitter van de Opleidings Commissie Technische Geneeskunde en lid van verschillende evaluatie- en kwaliteitscommissies. Daarnaast werkte hij als student-assistent bij de opleidingen Technische Geneeskunde, Biomedische Technologie en Geneeskunde. Tijdens zijn afstuderen aan de Universiteit Twente was hij één van de initiatiefnemers voor de oprichting van de beroepsvereniging voor Technische Geneeskunde (NVvTG) en was lid van het oprichtingsbestuur van 2008 tot 2011. Binnen deze bestuursfunctie was hij verantwoordelijk voor het organiseren van wetenschappelijke bijeenkomsten, de huidige beroepscode en het kwaliteitsregister voor Technisch Geneeskundigen.

Na het afronden van het artsexamen zal Reinoud in september 2013 beginnen met de geconcentreerde opleiding Tandheelkunde voor artsen (TOVA) waarna hij in 2015 zal starten met zijn opleiding tot medisch specialist in de Mondziekten, Kaak- en Aangezichtschirurgie (opleider prof. dr. S.J. Bergé, UMC St Radboud Nijmegen).

# LIST OF PUBLICATIONS

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- Sinus floor augmentation surgery using autologous bone grafts from various donor sites: A meta-analysis of the total bone volume.
  *Tissue Engineering Part B Reviews, 2010 16(3): 295-303*

- A meta-analysis of histomorphometric results and graft healing time of various biomaterials compared to autologous bone used as sinus floor augmentation material in humans.
  *Tissue Engineering Part B Reviews, 2010 16(5): 493-507*

- Predictive value of ridge dimensions on autologous bone graft resorption in staged maxillary sinus augmentation surgery using Cone Beam CT.
  *Clinical Oral Implants Research, 2012 23: (4) 409-415*

- Three different strategies to obtain porous calcium phosphate cements: Comparison of performance in a rat skull bone augmentation model.
  *Tissue Engineering Part A, Article published online: 31 JAN 2012*

- Maxillary sinus floor augmentation with injectable calcium phosphate cements: A pre-clinical study in sheep.
  *Clinical Oral Implants Research, Article published online: 15 FEB 2012*

- Maxillary sinus augmentation with micro structured tricalcium phosphate ceramic in sheep.
  *Clinical Oral Implants Research, 2012 23: (3) 274-280*